

Determining Cetacean – Cephalopod Trophic Interactions – An Isotope Approach

By

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DECLARATION

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ABSTRACT

Determining the biotic and abiotic influences on the distribution and abundance of marine mammals is essential for understanding the dynamics of the food chain. The predator-prey relationship can be deterministic in shaping both the community structure and function of marine ecosystems. This is especially pertinent to recovering toothed whale populations given their large size and high prey consumption rates. A greater knowledge of the trophic linkages between toothed whales and their prey will facilitate assessments of their combined impact on the ecosystem since marine food webs are a fusion of bottom-up and top-down energy and nutrient flow. This is of particular interest for regions that have recovering whale populations and varying climactic changes, such as Australia.

Whales and dolphins strand in all Australian coastal areas. However, it is the southern states, of which Tasmania is a particular hotspot, that experience frequent strandings. In the previous two decades there has been in excess of 70 mass strandings. Two of the most common species to strand are long-finned pilot whales *Globicephala melas edwardii* and sperm whales *Physeter macrocephalus*. Until 2010, there had been 3974 of these two species that had stranded around Tasmania, 87% of which were long finned pilot whales and 13% were sperm whales (parks.tas.gov.au). Despite the frequent stranding of these toothed whales there is a paucity of trophic information for these species from the Tasmanian region. Similarly, comparatively little is known of the trophic dynamics of oceanic cephalopods which are considered a major prey of many toothed whales in this part of the world.

This study used stable isotope analysis to quantify the diet and trophic relationship between toothed whales and cephalopods in regions surrounding Tasmania. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic analysis was conducted on cephalopods that were captured incidentally by commercial fisherman to provide a baseline with which to compare isotopic values of cephalopod prey from predator's stomachs. Isotopic values indicated that the cephalopod community was inclusive of 3 distinct trophic levels ($6.7 \pm 1.1 \text{ ‰}$) (*Moroteuthis*

ingens) to 12.0 ± 0.5 ‰ (*Idioteuthis cordiformis*), ranging from lower trophic crustacean feeders to higher trophic fish feeders. Some cephalopod species provided evidence of resource partitioning while other species indicated a dietary shift from lower to higher trophic levels as they matured. Furthermore, cephalopods occupy a range of trophic levels and are therefore important vectors in transferring energy up the food chain, particularly to toothed whales.

Intrinsic factors such as age, sex or lactation status exhibited little variation on skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of long-finned pilot whales from 3 stranding events off the coast of Tasmania. Nevertheless, small variations due to stranding events were evident. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggested that some adult pilot whales may have a more demersal or shelf foraging habitat while most reflect a pelagic oceanic foraging habitat. Whales showed little trophic enrichment compared to beaks from their stomachs suggesting supplementation of their predominantly teuthophageous diet with other organisms. Long-finned pilot whales also had one of the lowest $\delta^{15}\text{N}$ values (12.2 ± 0.4 ‰) for pelagic marine mammals in the region.

Isotopic analysis defined sperm whales as an apex predator in this region. However, based on skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, sperm whales showed low variation in foraging based on strandings. Sperm whales are largely teuthophageous feeding on oceanic squid from the meso- and bathy-pelagic zone. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of squid beaks from their stomach contents confirmed that the whales had been foraging in an analogous isotopic region to that of subtropical waters around Tasmania. The isotopic signature of sperm whales was likely a result of a mixture of both low and high trophic level cephalopod prey, with the $\delta^{15}\text{N}$ value of some prey (e.g. *M. hamiltoni* 16.8 ± 0.7 ‰) exceeding that of the $\delta^{15}\text{N}$ value of the predator (e.g. 14.7 ± 0.8 ‰).

Smaller-sized beaked whales had lower $\delta^{15}\text{N}$ values (range 11.0 ‰, *Cuviers cavirostris* calf to 13.2 ‰, *Tasmacetus shepherdi*) and assumed lower trophic position than some other odontocetes from the region. There was evidence of niche separation between species. Furthermore, isotopic values of stomach contents of a Cuvier's beaked whale suggested it might not be predominantly

teuthophageous. The beak $\delta^{15}\text{N}$ values of all cephalopods from the stomach contents exceeded the $\delta^{15}\text{N}$ values for the predator itself. Comparisons between different whale tissues of the same animal highlighted the importance of species-specific isotopic discrimination values to accurately evaluate foraging strategies.

Toothed whales are good biological samplers for describing unknown cephalopod assemblages from meso- and bathypelagic water masses. Combined isotopic analysis of stomach contents with that of the predator highlighted whether oceanic cephalopods were likely to be a dominant prey item in their diet. Moreover, the importance of cephalopods as mid and higher order predators in the region and their role in transferring energy up the food chain was confirmed. However, evidence suggested that the toothed whales themselves were more generalist rather than specialist foragers.

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CHAPTER ONE

GENERAL INTRODUCTION

THE TROPHIC ROLE OF APEX PREDATORS IN THE MARINE ECOSYSTEM

Determining biotic and abiotic factors that affect the distribution and population of marine mammal species is a critical component in ecological studies (Croll et al. 2005). Apex predators and large predators at the top of the food chain impact the structure and function of marine ecosystems by controlling prey density of smaller medium size or mid-trophic predators i.e. mesopredators (Wallach et al. 2015a,b). Although the important keystone function of apex predators within an ecosystem is well understood (Baker & Clapham 2004, Heithaus et al. 2008, Baum and Worm 2009, Estes et al. 2011), mesopredators who predate on smaller resource species also have a formative role in determining community structure (Heithaus et al. 2008, Wallach et al. 2015a). The absence or removal of apex predators and other top predators may result in reduced pressure on mesopredators. Greater abundance of these mid-level predators consequently exerts pressure on their prey (Strong et al. 2010). Alternatively, an abundance of mid-size predators may reverse the regulation on the food web exerted by apex predators and apply negative pressure on the survival of large apex predators that have become endangered due to overfishing or hunting (Strong et al. 2010). It is imperative that we also understand the functional position of organisms including mesopredators in addition to that of the apex predator to fully understand trophic interactions and community dynamics (Heithaus et al. 2008, Yick et al. 2012). For example, the removal of predatory fishes has led to their replacement by increasing numbers of invertebrates such as cephalopods, crustaceans and jellyfish (Caddy & Rodhouse 1998, Dulvy et al. 2003, Baum & Worm 2009). Moreover, the immense human impact on the food web through hunting, fishing and whaling has resulted in disproportionate losses of large predators at the top of the food chain (Strong et al. 2010). It is therefore feasible that the distribution and abundance of higher order or apex predators are useful as indicators of the ocean's resilience and sustainability (Ramos & González-Solís 2012).

Trophic cascades, which are the most straightforward top-down interactions, have been

gaining increased attention, accounting for a substantial volume of our knowledge base on food webs (Baum & Worm 2009, Young et al. 2015a). Top level and apex predators impact their associated ecosystems by exerting force from the top of the food chain and down through all trophic levels. This leads to a number of ecosystem effects as well as species effects (Estes et al. 2011). However, top-down effects do not act in isolation. Marine food webs are a fusion of bottom-up energy and nutrient flow as well as predators exerting top-down control on producers. Both top-down and bottom-up mechanisms in combination are essential components in modifying and structuring ecosystems (Baum & Worm 2009, Strong et al. 2010, Young et al. 2015a).

Both top-down and bottom-up processes are highly influenced by climatically driven fluctuations in marine ecosystems (Murphy et al. 2007a, Cutt et al. 2015). Climatic changes (bottom-up processes) have become an important factor to consider and understand in determining ecosystem predator-prey interactions and is complicated by human exploitation (top-down processes) (Murphy et al. 2012). Rapid warming of some regions may have large impacts on lower trophic levels due to its effect on phytoplankton and lower trophic organisms, which in turn affects foraging by higher trophic level organisms (Horswell et al. 2016). Furthermore, the exploitation and removal of large predators such as was evident with whales in the last two centuries has resulted in a severe decline in numbers of these predators worldwide. As larger predators became scarcer, resources are targeted further down the food chain with lower-trophic level species coming under exploitation, such as Antarctic krill *Euphausia superba* (Pauly et al. 1998, Ainley & Pauly 2014). Ecosystem modeling of the Scotia Sea, for example, has highlighted that the removal of large predators such as seals and whales over previous centuries, has likely created a long-term cascade effect with an alteration at the base of the food chain. Furthermore, a decline in krill abundance due to climatic changes is confounded by the top-down forces exerted by changes in foraging behavior of predators (Murphy et al. 2007b).

The trophic impact of cetaceans on ecosystems

During the 20th century whaling was most intense in the southern hemisphere with a 2.5 times greater number of whales killed than in the northern hemisphere (Rocha et al. 2015). In the Southern Ocean each species of whale, with possibly the exception of the minke whale *Balaenoptera acutorostrata*, was reduced to 5-10% of pre-exploitation

numbers (Croxall et al. 1992). Since food seems to be a dominant regulatory factor in populations (Croxall et al. 1992) it is suggested that the removal of whales may have boosted the availability of krill to other Southern Ocean predators, in the vicinity of 150 million tonnes per year (Laws 1977). An increase in populations of krill-eating penguins and seals as well as minke whales during the 1950s and 1960s has resulted in the generally accepted 'surplus krill hypothesis' (Croxall et al. 1992). With a subsequent moratorium worldwide on industrial whaling in the 1980s (Rocha et al. 2015) there may likely be a subsequent top-down influence on the dynamics of the ecosystem.

The large prey consumption of recovering whale populations from commercial hunting in the 19th and first part of the 20th century may be expected to exert top-down ecosystem effects. For example, ecosystem modeling revealed that predation by recovering sperm whale *Physeter macrocephalus* populations may potentially result in a decrease in rockfish biomass in the northeast Pacific food web, with a consequential cascading effect for some demersal fish species (Surma & Pitcher 2015). However, many of the negative top-down effects of whale recovery are tempered by variable primary productivity, potentially overcoming many cascading trophic effects. Although not all effects could be removed, an increase in primary productivity, possibly resulting from increased nutrient recycling and varying oceanic temperatures, counteracted some cascading effects likely due to the subsequent greater abundance of food resources at multiple trophic levels (Surma & Pitcher 2015).

While modeling of whale recovery has exhibited an effect on some ecosystems, there has been little support for fisheries being heavily impacted (Surma & Pitcher 2015). Nevertheless, in some parts of the world large whales are seen as possible competitors with humans for food resources. This has culminated in a proposal that whales theoretically should be culled so as to ensure fisheries' sustainability (Morrisette et al. 2010). No real evidence has been found for this theory 'whales eat fish' (Morrisette et al. 2010). On the contrary, ecosystem models have highlighted how multidimensional the outcomes of a reduction in large predators within a marine pelagic ecosystem would be (Surma et al. 2014). Worldwide, marine mammals including whales are mostly foraging on different food resources than those targeted by fisheries (Morrisett et al. 2010, 2012) or are unfit for human consumption such as deepwater, ammoniacal squid (Clarke 1980). In fact, toothed whales in the Caribbean, for example, were more likely

affected by fisheries than vice versa (Morrisett et al. 2010). Moreover, the primary production needed to sustain the prey of the marine mammals was lower than that needed or removed by fisheries. Similarly, the overall trophic level of prey was lower than that removed by fisheries (Morrisett et al. 2010, 2012). Bayliss et al. (2015) concluded that the greatest influence on the biomass of some predators, such as southern sea lions, is the bottom-up forcing on lower trophic small prey such as plankton, fish and cephalopods. Furthermore, ecosystem modeling underscored that the removal of large predators, such as rorquals, from the pelagic ecosystem may provide some release of top-down pressure but result in negative impacts on bottom-up forcing affecting consumers across the whole food web (Surma et al. 2014, Bayliss et al. 2015).

FORAGING ECOLOGY OF APEX PREDATORS

Foraging theory

Determining the causal factors of ecosystem change as well as their impact potential on the marine environment requires a comprehensive understanding of predator-prey trophic interactions (Ramos & González-Solís 2012). It is therefore essential to understand the foraging dynamics of iconic marine mammals. Optimal foraging theory suggests that large air-breathing marine predators would weigh up the costs of their foraging in terms of food required and the effort needed to acquire it while concurrently maximizing the benefits (Spitz et al. 2011, Tyson et al. 2016). The main pursuit of an organism is to obtain enough energy from their prey to maintain their basal metabolic rate in addition to other activities such as foraging and reproduction (Spitz et al. 2011). The energy required by deep-diving cetacean species is largely dependent on their metabolic rate and cost of living since diving for prey affords a complex array of physical challenges for optimizing foraging success (Friedlaender et al. 2016). Consequently, changes or fluctuations in prey will more likely affect those cetaceans that have higher energetic requirements (Spitz et al. 2011). Lambert et al. (2014) predicted the foraging habitat of cetacean species and the distribution of their prey based on their energetic needs. Cetaceans with higher energy requirements such as delphinids and globicephalids foraged in habitats of high productivity or prey biomass. Moreover, Lambert et al. (2014) also found that the diving abilities of various species correlated with their preferred foraging habitat. Shallow diving delphinids that had the highest cost of living were associated with greater prey aggregations in the upper layers

of the water column (Lambert et al. 2014). Conversely, sperm whales and beaked whales that had the lowest cost of living foraged in deeper oceanic layers reflecting their greater diving ability (Baird et al. 2006, Tyack et al. 2006, Lambert et al. 2014).

Importance of cephalopods as dietary prey

Cephalopods, squids in particular, are a significant prey resource for many fish, seabird and marine mammals (Clarke 1996, Collins & Rodhouse 2006, Rodhouse 2013, Young et al. 2015a, Xavier et al. 2016). Industrial whaling provided a rich source of dietary data from large predators from various regions of the world (e.g. Clarke & MacLeod 1976, 1980, Martin & Clarke 1986, Clarke et al. 1993) including the Southern Ocean and Antarctica (Laws 1977, 1980, Clarke 1983). This highlighted the fact that these large whale predators were eating a diverse range of squid species as well as an enormous biomass of squid. Crude estimates of prey/squid biomass (Laws 1977, Clarke 1983) were facilitated by species identification based on beak morphology from stomach remains (Clarke 1980). However, once the moratorium on whaling was introduced in the 1980s in Australia, access to data from the whaling industry was no longer available (Suter 1982). These broad regional studies were foundational to our understanding of cephalopods as significant prey for whales.

Many cetaceans were identified as primarily teuthophagous (Rodhouse 2013) with over 80 % of toothed whales frequently consuming cephalopods (Clarke 1996). The stomach contents of some sperm whales stranded in Tasmanian waters have contained as many as 50 cephalopod species (Evans & Hindell 2004a). Based on the worldwide sperm whale population a conservative estimate of annual cephalopod consumption is in the order of 100 million tonnes. However, approximations of total annual cephalopod consumption (predominantly oceanic) in the Antarctic Ocean range from 12.5 to 24 million tonnes (Santos et al. 2001a). As whale populations continue to recover from commercial hunting the demand for cephalopods as a prey resource may increase resulting in increased competition with pinnipeds, seabirds and other marine top predators (Surma & Pitcher 2015).

Ecologically, squids occupy mid to high trophic positions in the food web (Navarro et al. 2013) usually at trophic levels two to four across different ecosystems (Coll et al. 2013). They likely account for a large percentage of biomass in marine communities (Coll et al.

2013). Additionally, squid inhabit a diverse array of ecological niches as evidenced by their ubiquitous presence from the poles to the equator, including neritic to oceanic waters, and from abyssal depths to the epipelagic (Rodhouse 2013). Squid populations are characteristically unstable (Rodhouse, 2001) and extremely plastic in their response to varying environmental conditions (e.g. Villanueva 1992, Hatfield 2000, Ichii et al. 2004, McGrath-Steer 2004, Pecl et al. 2004). Their voracious appetite and opportunistic feeding behaviour enables them to rapidly exploit favorable conditions (Rodhouse 2001). Furthermore, they are able to exploit potential prey resources across the whole food web including zooplankton and crustaceans as well as fish and squid (Navarro et al. 2013). Coll et al. (2013) revealed that squid might be keystone species exerting strong top-down control on their prey while simultaneously representing a significant prey resource for apex predators. While there may be strong interrelationships with neritic squid as both predator and prey in inshore regions, in oceanic waters and high nutrient areas squid may be expected to exert the greatest impact from the bottom up on their predators (Coll et al. 2013). Ecosystem models reveal that a removal or decrease in squid was more likely to negatively impact megafauna such as cetaceans but have a positive effect on squid prey (Coll et al. 2013).

Apex predators as biological samplers

Large deep-diving oceanic apex and higher order predators are ideal biological samplers of cephalopods that are logistically difficult to sample (e.g. Cherel et al. 2009a, Xavier et al. 2014, Negri et al. 2016, Seco et al. 2016). An examination of cephalopods from stomach contents of deep-living predators or predators foraging on deep oceanic cephalopods can provide information on rarely observed species thus enhancing knowledge of both distribution and abundance (Xavier et al. 2002, Xavier et al. 2006, Cherel et al. 2009a, Hoving et al. 2014, Liu et al. 2015). Cherel et al. (2009a) documented the deep-sea cephalopod assemblage from the Bay of Biscay providing evidence of a previously unknown trophic structure between species. While methods such as telemetry and satellite tracking are logistically viable for obtaining important foraging information on large predators, using the predator themselves as a biological sampler can further provide data on trophic interactions, critical for conservation and management of both the predator and prey (Weimerskirch et al. 2005, Walters 2013, Xavier et al. 2014, Hoving et al. 2014, Surma & Pictcher, 2015, Guerreiro et al. 2015).

Dietary methods - general

Traditionally, dietary analysis has been undertaken utilizing stomach contents of individual cetaceans taken as by-catch, from strandings, or acquired through commercial fisheries of selected species (MacLeod et al. 2003, Rodhouse 2013). Additionally, much of the early information on diet was provided through access to cetaceans from industrial whaling (Clarke 1980, Clarke 1996). Although this method has afforded foundational data for understanding predator-prey dynamics, it is not without its own unique assemblage of advantages, disadvantages and limitations (Young et al. 2015a). One of the disadvantages most pertinent to cetacean studies is the fact that it is only a snapshot in time of the predator's diet (MacLeod et al. 2003). This is a particular drawback for stranded animals that may not be healthy specimens. In a study on 405 marine mammals that stranded on Cape Cod and southeastern Massachusetts, disease affected 37% of the cases that could be assigned a cause of death (Bogomolni et al. 2010). However, disease or sickness that may disrupt the normal foraging habit of animals is unlikely to be a significant issue for dietary analysis of stomach contents in mass stranded individuals. Bogomolni et al. (2010) found that the death of 92% of necropsied animals from mass strandings was not attributable to disease but to stress and factors relating to the stranding event itself. The authors further suggest that disease is not the driving factor in stranding events for that region but rather due to some kind of natural non-pathological reason. In Tasmanian waters, the tracking of mass stranded long-finned pilot whales *Globicephala melas edwardii* that have been rescued and successfully released back into the sea (Gales et al. 2012), further indicate the relative health of individuals that have mass stranded. The survival rates were high with the stranded whales reuniting after release, which is consistent with their known social behavior (Gales et al. 2012). There is a large amount of speculation regarding the reason for stranding events (Bradshaw et al. 2006). It has been suggested that these highly social oceanic species may inadvertently end up in coastal waters due to a climactic event such as a storm or from following prey inshore or a sick individual from their pod (Evans et al. 2005). They then may become confused or disorientated by the topography of the shallower waters (Bogomolni et al. 2010).

Another limitation of stomach content analysis is the differential digestion of prey, particularly of soft-bodied organisms, given the acidic conditions in the stomach of

marine mammals is likely to cause sampling bias (MacLeod et al. 2003, Pierce et al. 2004). Hard parts such as cephalopod beaks are preferentially retained over otoliths resulting in an over or under estimation of one prey type over another (squid and fish respectively) (Bowen & Iverson 2013). For example, a large amount of beaks retained in a cetacean's stomach may have accumulated over time overestimating their importance, whereas fish would be digested relatively quickly and as a result not considered an important prey item (Pierce et al. 2004). As a consequence, researchers studying trophic ecology of marine predators have embraced the relatively more recent biochemical techniques based on the idea that 'you are what you eat' using stable isotopes (Jackson et al. 2007).

Stable isotopes

The previous decade has seen persistent growth in the use of biochemical tracer techniques such as stable isotope analysis (Crawford et al. 2008, Newsome et al. 2010, Layman et al. 2012). This is most likely due to the fact that this approach offers a number of advantages over the traditional stomach content analysis. Isotope analysis is based on the theory that prey is ingested and assimilated into the predator's tissue, whereas gut analysis is restricted to recently ingested prey remains (Young et al. 2015a). Retrospective dietary analysis is possible based on both hard (e.g. teeth, bone and hair) and soft (e.g. blood, liver, muscle) tissues representing time periods from days to months to years (Newsome et al. 2010, Xavier et al. 2011, Xavier et al. 2015, Young et al. 2015a). Two of the most common stable isotopes used to date in trophic ecology are carbon and nitrogen (Crawford et al. 2008). These elements are predominantly acquired through protein consumption and water intake and are subject to predictable changes with trophic transfer within a food web (Borrell et al. 2013a). As a result, isotopic analysis can be used to investigate foraging due to the isotopic values of marine organisms being directly linked to those of their prey (de Niro & Epstein 1978, 1981). Nevertheless, one of the greatest limitations of stable isotope analysis is its inability to identify prey to species level (Young et al. 2015a). Furthermore, caution is needed in the interpretation of directly relating the isotopic signature of the predator to a particular region, due to the potential of the predator feeding on offal or discards from fishing boats (Votier et al. 2008). However, many deep foraging oceanic whale predators appear to feed in waters not targeted by fisheries.

Carbon isotopes

Carbon stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$) can be utilized as dietary tracers of varying carbon sources within a marine trophic web or across ecosystems (Crawford et al. 2008, Layman et al. 2012). The $\delta^{13}\text{C}$ values of primary productivity or particulate organic matter (POM) at the base of the food chain track fluctuations in the ocean's productivity. Since there is very little variation in $\delta^{13}\text{C}$ values reflected in consumers with trophic transfer (i.e. $\sim 1\text{‰}$), $\delta^{13}\text{C}$ values provide a signature of the consumer's foraging area (de Niro & Epstein 1981, Kurle & Worthy 2002, Cherel & Hobson 2007, Newsome et al. 2010, Layman et al. 2012). Spatial variability in productivity results in higher $\delta^{13}\text{C}$ values for inshore high productivity areas as opposed to lower $\delta^{13}\text{C}$ values in offshore low productivity areas (Newsome et al. 2010). Similarly, pelagic/benthic gradients are also evident with the baseline of pelagic food webs being less productive and therefore less enriched in ^{13}C than benthic food webs. Therefore, $\delta^{13}\text{C}$ values are able to delineate between inshore/offshore and pelagic/benthic contributions to dietary intake (Hobson et al. 1994, Cherel & Hobson 2007), as depicted below:



The $\delta^{13}\text{C}$ values of primary producers and POM also vary predictably with latitude (Newsome et al. 2010). At lower latitudes ^{13}C is more enriched compared to ^{13}C at higher latitudes (see Cherel et al. 2007). As a result, latitudinal $\delta^{13}\text{C}$ values have been effectively used to define foraging habitats and migration patterns in predators when the signature of the consumer is compared to isotopically distinct spatial and geographical areas (Kurle & Worthy 2002, Mendes et al. 2007a, Cherel & Hobson 2007, Cherel 2008).

Nitrogen isotopes

Trophic relationships within marine ecosystems can be estimated using nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) (Kelly 2000). Unlike ^{13}C , which shows little variability with trophic transfer, ^{15}N has a predictable stepwise enrichment between predator and prey with trophic transfer (Minigawa & Wada 1984, Vanderklift & Ponsard 2003). The ^{15}N enrichment between each trophic level is expected to range between 2 - 5 ‰ (Hobson & Clark 1992, Kelly 2000, Post 2002). Consumers are usually enriched in ^{15}N relative to their prey because of the loss of the lighter ^{14}N isotope during excretion (Vanderklift & Ponsard 2003). Consequently, $\delta^{15}\text{N}$ values can be used as a proxy to determine a predator's trophic position in a food web as well as its relative position and relationship to other organisms in an ecosystem (Kelly 2000, Ruiz-Cooley et al. 2004, Herman et al. 2005). Studies using $\delta^{15}\text{N}$ values have been able to document niche overlap between predators as well as competition for prey resources (e.g. Auriolles-Gamboa et al. 2013, Navarro et al. 2013, Staudinger et al. 2014). Natural variation in nitrogen isotopic composition of seawater between geographical areas can be greater than trophic enrichment. Therefore, comparisons between ecosystems require knowledge of the baseline $\delta^{15}\text{N}$ value (Navarro et al. 2013) or of organisms at the base of the food chain in that region such as zooplankton or phytoplankton (Post 2002).

Turnover and discrimination rates of isotopes

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reflected in a consumer are species and tissue specific (Borrell et al. 2013b, Cherel et al. 2015). The combined effects of metabolism (a balance between anabolism and catabolism) along with growth rate (time required for tissue renewal) help regulate the isotopic values expressed in various tissues of marine mammals and other organisms (Fry & Arnold 1982). Therefore, multiple isotopic pools with differing turnover rates are likely to exist within the same animal (Newsome et al. 2010). Tissues vary in their turnover rate with more metabolically active tissues like liver and blood having a higher turnover rate than tissues with a lower metabolic rate such as muscle and collagen. Therefore, consumer tissues with a faster, more active turnover rate will incorporate the signature of their prey over a shorter time period relative to tissues with a slower metabolic turnover rate (Tieszen et al. 1983, Hobson & Clark 1992). Studies that have evaluated different tissues with varying incorporation rates obtained from the same animal simultaneously have been able to document

trophic foraging over a larger time scale (e.g. MacNeil et al. 2005). Similarly, tissues that grow continuously but are metabolically inert, such as marine mammal teeth or whiskers, can provide retrospective dietary analysis based on the growth of the tissue (Mendes et al. 2007a, b, Newsome et al. 2009, Newland et al. 2011). Due to the differing turnover or incorporation rates of food into the consumer's tissues there is a resulting variation in the isotopic values between the food of the consumer and the consumer's tissues. The difference is called the discrimination factor (Bond & Hobson 2012, Cherel et al. 2005, Greer et al. 2015, Cherel et al. 2015). Utilizing species and tissue specific discrimination values, resulting from variations in turnover rate are critical when comparing isotopic values of potential prey to predator tissues (Horstmann-Dehn et al. 2012, Browning et al. 2014). However, determining discrimination factors is time consuming and not always logistically viable since it mostly requires an animal to be fed a known diet under captive conditions (e.g. Browning et al. 2014, Giménez et al. 2016).

RESEARCH RATIONALE AND STRUCTURE

The rationale for this study was to assess and explore the trophic relationship between selected cetaceans and cephalopods from Tasmanian waters, off southeastern Australia. Although Tasmania is a hotspot for whale strandings there is a paucity of documented information on the foraging ecology for many toothed whale species from this region. Toothed whales in the region range from the smaller beaked whales to the larger more iconic sperm whale. Almost half of all known cetacean species occur in the Australasian region. Odontocetes or toothed whales are particularly prone to stranding in the southern regions of Australia with sperm whales and long-finned pilot whales accounting for a large percentage of mass strandings (Gales et al. 2013). Until 2010, there had been 3974 individuals of these two whale species stranded in Tasmanian waters, of which 87% were long finned pilot whales and 13% were sperm whales (parks.tas.gov.au).

Oceanography around Tasmania

The island state of Tasmania off the southeast Australian mainland (located at 39-43°S latitude) is a region highly affected by environmentally induced changes in marine primary productivity (Watson et al. 2012). In any ecosystem the dominant driving force influencing primary productivity are nutrient fluctuations (Prince 2001). In waters surrounding Tasmania perhaps the most influential source of nutrients is the deep

oceanic subantarctic waters (SAW). The SAW mass typifies that found in most temperate regions of the world as it is characterised by a spring and autumn bloom resulting in seasonally mixed layers. However, the south west of Tasmania is occasionally affected by the Zeehan Current (ZC), which is an extension of the surface flowing Leuwin Current, bringing intrusions of oligotrophic subtropical waters (Harris et al. 1991, Prince 2001). Moreover, waters off eastern Tasmania are governed by a complex hydrography whereby the phytoplankton biomass is a result of seasonal and sporadic events (Harris et al. 1987, Young et al. 1996a). The warm stratified nutrient depleted East Australian Current (EAC) brings southward incursions to eastern Tasmanian waters which when intensified (i.e. in strength, duration and frequency) can result in modifications of the marine ecosystem (Pitt et al. 2010, Johnson et al. 2011). The nutrient poor EAC then encounters the cooler, frequently deeply mixed, nutrient rich SAW that forms the Subtropical Convergence (STC) (Harris et al. 1991, Young et al. 1996b, Prince 2001). The STC, a broad region of enriched oceanic productivity is also subject to both seasonal and annual latitudinal shifts around the southern and eastern regions of Tasmania resulting in fluctuations in productivity (Harris et al. 1987, Young et al. 1993, Prince 2001).

Study Species

Cephalopods in Tasmanian waters

Worldwide, cephalopods are key prey items for many marine higher trophic species. Predators inhabiting waters surrounding the island state of Tasmania, off southern Australia are no exception. In this marine sector, cephalopods are prey for birds (e.g. Gales & Pemberton 1990, Weimerskirch & Cherel 1998, Hedd & Gales 2001), fish (e.g. Hallet & Daley 2011, Pethybridge et al. 2011) and marine mammals (e.g. Gales & Pemberton 1994, Evans & Hindell 2004a, Arnould et al. 2011). Despite the importance of cephalopods in the diet of predators in the region, there is a relative lack of data available on deepwater oceanic species, particularly as a prey resource (although see Pethybridge et al. 2010 for lipid and mercury profiles). Currently most cephalopod research has targeted the two most common ommastrephids in this region (e.g. *Nototodarus gouldi*, Jackson et al. 2003, Stark et al. 2005; *Todarodes filippovae*, Jackson et al. 2007, Kojadinovic et al. 2011, Pethybridge et al. 2012, 2013) as well as neritic species (e.g. *Sepioteuthis australis*, Jackson & Pecl 2003, Pecl & Moltchaniwskyj 2006,

Smith et al. 2015; *Octopus tetricus*, Ramos et al. 2015; *Octopus maorum*, Grubert et al. 1999). The lack of research on deepwater oceanic cephalopods is largely due to logistical difficulties associated with sampling these species.

Long-finned pilot whales

Distribution and biology

Long-finned pilot whales (*Globicephalus melas edwardii*) are mid-sized toothed whales of the suborder odontocete, belonging to the family Delphinidae, of which there are approximately 35 species (Martin & Reeves 2002). They are distributed anti-tropically in the cold temperate and subpolar waters of the northern and southern hemisphere. However, the distribution is more widespread for the southern hemisphere subspecies *G. melas edwardii* that has a circumantarctic distribution as far south as the Antarctic convergence (<http://www.iucnredlist.org>, Oremus et al. 2009, Santos et al. 2010, Mansilla et al. 2012, Fullard et al. 2016). They are a sexually dimorphic species with a maximal size of at least 5.5 m for females and 6.3 m for males (Martin et al. 1987, Bloch et al. 1993). Female lifespan may reach 60 years whereas males may live up to 46 years (Bloch et al. 1993). Female sexual maturity is reached at seven years and at a length of 3.4 m, while males mature at a greater age and length (9 - 17 years and 5 m length respectively) (Sergeant 1962, Martin et al. 1987, Desportes et al. 1994.)

Foraging behaviour

Long-term relationships are evident in this highly social odontocete, often being found in groups as small as two but as many as 1000 individuals (Ottensmeyer & Whitehead 2003, de Stephanis et al. 2008). They tend to forage in groups at night adjusting the time spent foraging to seasonal night length (Giacome et al. 2016). Prey is acquired through deep diving activity with dives recorded from 360 m (Baird et al. 2002) to over 800 m (Heide-Jørgensen et al. 2002).

Long-finned pilot whales are generally assumed to be an oceanic species inhabiting the deeper waters off the continental edge and shelf (Gales & Pemberton 1992, Monteiro et al. 2015a). Nevertheless, Goetz et al. (2015) determined that Iberian Atlantic pilot whales inhabit waters where fisheries target shelf fish species thus suggesting that pilot whales may forage not only in oceanic waters but also in neritic waters (see also Beatson et al. 2007a, b, Beatson & O'Shea 2009, Spitz et al. 2011, Mèndez-Fernandez et

al. 2012). Although long-finned pilot whales have been recorded as having a preference for foraging on cephalopod prey in many parts of the world (Gales & Pemberton 1992, Gannon et al. 1997, Santos & Haimovici 2001, de Pierrepont et al. 2005, Beatson et al. 2007b, Beatson & O'Shea 2009, Mansilla et al. 2012, Santos et al. 2014) fish have likewise been a significant prey item in some regions (Overholtz & Waring 1991, Spitz et al. 2011). Moreover, the low energy density of cephalopods may trigger the consumption or supplementation of higher energy dense fish if prey is scarce or fat reserves are depleted in the whales (Lockyer 2007).

Sperm whales

Distribution and biology

Sperm whales *Physeter macrocephalus* have a worldwide distribution in deep oceans from the equator to the poles (Gosho et al. 1984, Alexander et al. 2016). Concentrations of sperm whales may often be associated with oceanic features such as high secondary productivity as well as steep marine landscapes (Jacquet & Whitehead 1996). *M. physeter* are the largest of all odontocetes. They exhibit extreme sexual dimorphism with recorded lengths up to 18 m for males (Allen 1980) and 11 - 12 m for females (Rice 1989 cited in Evans 1997). Evans et al. (2004b) recorded a maximum age of 64 years for females up to 12 m in length.

M. physeter demonstrate sex-biased dispersal with females showing a tendency towards philopatry compared to males that disperse from the natal pod as they mature (Alexander et al. 2016). Males disperse from the natal pod at around 9 - 10 years that correlates with puberty (Mendes et al. 2007b), becoming more solitary as they mature. Mature males do not form coalitions with other males and are rarely territorial (Cooke & Whitehead 2004). Females, on the other hand form long-term social bonds in groups that also comprise juveniles of both sexes (Christal et al. 1998). The female social groups are confined to the tropical and temperate waters of the lower latitudes, rarely travelling south of 40° S (Dufault et al. 1999) whereas mature males extend their range into polar waters (Gosho et al. 1984, Mendes et al. 2007a, b). Mature males only return to the lower latitudes where the females reside to mate (Gosho et al. 1984).

Foraging behaviour

Sperm whales are a highly mobile species with migration related to feeding success (Whitehead 1996). Numerous cephalopod beaks have been found in sperm whale stomachs leading to the assumption they are a teuthophageous predator in deep oceanic waters (Clarke 1980, Santos et al. 2002, Evans & Hindell 2004a, Garibaldi & Podesta 2014, Harvey et al. 2014). With the exception of perhaps some of the larger beaked whales, sperm whales dive deeper for their prey than all other cetaceans (Martin & Reeves 2002). Their deep dives of up to 1860 m enable them to forage for mesopelagic and bathypelagic prey (Watwood et al. 2006, Davis et al. 2007, Teloni et al. 2008). Evans & Hindell (2004a) documented significant individual variation but with a high diversity of cephalopod prey in the diet of sperm whales off Tasmania.

Beaked whales***Distribution and biology***

Beaked whales (family Ziphiidae) are medium-sized cetaceans that make up about one fourth of all cetacean species. Second to delphinids they are one of the most speciose of all cetaceans, with currently 22 species relating to six genera *Hyperoodon* (two species), *Mesoplodon* (15 species), *Ziphius* (one species), *Indopacetus* (one species), *Berardius* (two species) and *Tasmacetus* (one species) (MacLeod et al. 2003, Dalebout et al. 2004, Thompson et al. 2016). However, data is sparse on many beaked whale species. This is probably a result of their deep-diving behaviour as they spend more than 80 % of their time at depth only surfacing for no more than an hour at a time (Rommel et al. 2006). For many regions of the world the incidence of beaked whales appears to be associated with the seabed topography i.e. slopes, canyons, escarpments and oceanic islands (MacLeod & Amice 2006). One of the most well known beaked whale species is *Ziphius cavirostris* Cuvier's beaked whale. It has a cosmopolitan distribution from the equator to the poles (MacLeod et al. 2006a). Conversely, perhaps the least known of the beaked whales is the Shepherd's beaked whale (*Tasmacetus shepherdi*) (Best et al. 2014) notwithstanding the fact that it likely has a circumpolar distribution in deep temperate waters of the southern hemisphere (Sekiguchi et al. 1996, MacLeod et al. 2006a, Pitman et al. 2006). Tasmania and southeast Australia have been identified as one of 23 key areas for beaked whales worldwide. There are 10 species recorded from the area making it the second highest region for diversity (MacLeod & Mitchell 2006).

Foraging behaviour

Foraging behaviour is probably one of the more studied subjects of these poorly understood species. This is likely due to the availability of deceased specimens for stomach content analysis resulting from strandings, by-catch or small fisheries (MacLeod et al. 2003). They are thought to be generalist feeders predominantly foraging on deepwater oceanic squid, fish and crustaceans with some species having a preference for one over the other (Santos et al. 2001b, MacLeod et al. 2003, Santos et al. 2007, Spitz et al. 2011, Wenzel et al. 2013). This is evidenced by the deep diving behaviour of beaked whales with foraging usually occurring at night (Au et al. 2013). Dives have been reported from tagged Cuvier's whales to around 3000 m making it one of the deepest and longest diving marine mammals (Schorr et al. 2014). It is likely that different species inhabit different ecological niches thus allowing them to coexist without competing for resources (MacLeod et al. 2003).

Aims

Elucidating the effects of bottom-up or top-down processes on the marine ecosystem requires a comprehensive understanding of the trophodynamics of apex and upper trophic level predators. Dietary data of higher order predators may be formative in determining the structure of the marine ecosystem due to their sizeable consumption rates. The distribution of a predator is often correlated with the abundance and distribution of its preferred prey. Dietary analysis of top predators will increase the understanding of these marine trophic linkages. This research on the diet of toothed whales is pertinent to understanding whale population recovery from commercial hunting in the late 19th to early 20th century.

Cephalopod beaks are often found in large numbers in toothed whale stomachs. Nevertheless, their contribution as an important prey resource in some instances has increasingly been questioned. Over the course of this study, Tasmania had a commercial deepwater fishery operating off the coast and provided a unique opportunity to obtain fresh specimens of squid species that would be otherwise difficult to obtain. The abundance of whale strandings in Tasmania further provided an archive of samples from deep diving oceanic whales that are logistically difficult to study *in situ*. Using this unique sample set, the objective of this research was to determine the importance of the

cetacean-cephalopod trophic relationship and subsequently infer regional resource partitioning using stable isotope analysis.

Thesis structure

This thesis is comprised of four data chapters that have been written as discrete manuscripts. As a result there is a small amount of textual repetition in the methodology and discussion sections between the chapters. These chapters focus on the predator-prey relationship for various cetacean species in the Tasmanian region, particularly as it relates to cephalopods. The objectives as they pertain to each chapter are described below. The cetacean-cephalopod relationship is synthesized in a final discussion chapter, followed by a reference list for the whole thesis.

Chapter objectives

Chapter 2

Very little is known about the community structure of cephalopods from Tasmanian waters. Therefore, an isotopic analysis on commercially jigged or trawled cephalopods from inshore pelagic and deep waters obtained from local commercial fishermen was conducted to determine the habitat signature of specimens caught in this region. Moreover, isotopic analysis was used to determine the trophic structure of the cephalopod assemblage in these waters. These isotopic values were then used as comparisons for beaks collected from stranded whale stomachs.

Chapter 3

This chapter focussed on long-finned pilot whales that have a high mass stranding rate in waters off the coast of Tasmania. However, there is very little information on their foraging ecology for this region. Isotopic analysis was used to determine if their foraging was influenced by intrinsic factors such as age, sex or lactation status. Furthermore, since strandings occurred in different locations and years, stranding effects were investigated to delineate if there were changes or variability in foraging. A previous study using fatty acid analysis found that long-finned pilot whales (thought to have a mixed diet of fish and squid) had a predominantly myctophid prey signature. Isotopic analysis from the cephalopod community study in Chapter two as well as from the beaks from the stomach contents of the whales were used to help clarify the dietary fish versus squid conundrum.

Chapter 4

Samples from mass strandings of sperm whales in Tasmanian waters were used to ascertain if foraging differed over time or space for this species. A previous study using stomach content analysis on sperm whales stranded in Tasmanian waters has revealed that they forage on a large diversity of cephalopods, greater than reported for other regions of the world. In this chapter, data on isotopic analysis on cephalopod beaks from the whale stomachs were used as biological samplers to further provide information on the cephalopod community structure in this region. A comparison of isotopic values of cephalopod beaks from the stomach contents with the skin from whales was used to determine if they were indeed a predominantly teuthophageous predator. Isotopic values of cephalopod beaks from the cephalopod fisheries samples (Chapter 2) were used as isotopic references for values of cephalopod beaks from whale stomachs to estimate the foraging habitat of the whale.

Chapter 5

Most beaked whales species (family Ziphiidae) are elusive and primarily strand singly. Isotopic analysis was used to provide further information on the trophodynamics of these seldomly observed whales. Comparisons of beaked whale isotopic values with other cetaceans were used to determine their trophic position in the Tasmanian region. Isotopic analysis of the squid beaks from a Cuvier's beaked whale helped elucidate whether this species is likely to be teuthophageous. A comparison of isotopic values from a number of whale tissues was analysed to complement existing foraging information for individual animals and species.

CHAPTER TWO

STABLE ISOTOPES DOCUMENT THE TROPHIC DYNAMICS OF CEPHALOPODS IN TASMANIAN WATERS

Abstract

Cephalopods are key organisms in the marine food chain serving as both voracious predators and a significant food source for other predators. Despite their importance there is a paucity of information on their trophic dynamics, particularly in some regions. The role of cephalopods were examined in a marine community in Tasmanian subtropical waters, off southeastern Australia, by examining the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the lower beaks of animals captured incidentally by commercial fisherman. Beak $\delta^{15}\text{N}$ values exhibited a graduating continuum over almost 2 trophic levels, ranging from $6.7 \pm 1.1 \text{ ‰}$ (*Moroteuthis ingens*) to $12.0 \pm 0.5 \text{ ‰}$ (*Idioteuthis cordiformis*). Based on $\delta^{15}\text{N}$ values, the cephalopods ranged from lower trophic level crustacean feeders (secondary consumers) to higher trophic level fish feeders (quaternary consumers). Size was correlated with $\delta^{15}\text{N}$ values for a subset of species indicating a dietary shift from lower to higher trophic levels as they matured. *I. cordiformis* was the most enriched in ^{15}N in this study, with corrected $\delta^{15}\text{N}$ isotopic values (15.8 ‰) exceeding that of the sperm whale *Physeter macrocephalus* (14.6 ‰), a top predator in this region. While 3 *Histioteuthis* species showed little variation in the $\delta^{13}\text{C}$ values of their beaks, $\delta^{15}\text{N}$ values were all distinctly different (*H. miranda* $11.7 \pm 0.8 \text{ ‰}$, *H. atlantica* $10.1 \pm 0.5 \text{ ‰}$, *H. macrohista* $8.3 \pm 0.9 \text{ ‰}$), providing evidence of resource partitioning among the species. There was a narrow range in beak $\delta^{13}\text{C}$ values ranging from $-18.6 \pm 0.6 \text{ ‰}$ for *M. ingens* to $-16.7 \pm 0.3 \text{ ‰}$ for *I. cordiformis* in the Tasmanian community as a whole. A comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between beaks and soft tissues for 6 cephalopod species showed that on average, beaks relative to mantle, were slightly enriched in ^{13}C (0.7 ‰) but highly impoverished in ^{15}N (3.8 ‰). This suggests that the $\delta^{15}\text{N}$ values of beaks need to be corrected when comparing with the muscle of other cephalopods or organisms. This study enhances our understanding of the trophic dynamics of cephalopods in Tasmanian waters. It also corroborates other findings that cephalopods are key components in many food webs including subtropical waters, occupying a range of trophic levels and are an important medium by which energy is transferred up the food chain.

INTRODUCTION

Cephalopods are a significant component of many marine ecosystems. They are found in virtually all of the major marine environments from the poles to the tropics and from the shallow continental shelves to the deep ocean. Throughout these environments they play an important role as both predator and prey, occupying mid to high trophic levels in marine food chains (Coll et al. 2013). Pivotal to the management and conservation of cephalopod predators as well as cephalopod prey is the ability to quantify trophic relationships. Primarily, this is due to the distribution of preferred prey species correlating with the movement and distribution of the predator, in addition to the abundance of a predator within a specific habitat (MacLeod et al. 2003). Quantifying trophic relationships is of particular relevance in regard to cephalopods since squid populations are characteristically unstable (Rodhouse 2001) and extremely plastic in their response to varying environmental conditions (e.g. Jackson & Domeier 2003, Ichii et al. 2004, Pecl & Jackson 2008, Hoving et al. 2013). A rapid turnover in generations with a lifespan less than a year for many species (see reviews Arkhipkin 2004, Jackson 2004) in concert with a cephalopod lifestyle defined by rapid, indeterminate growth (Jackson & O'Dor 2001) are key contributing life-history traits to this plasticity (Boyle & Boletsky 1996). Due to these features, environmental conditions can greatly influence growth rates and biomass over a fairly short time period thus significantly impacting predator or prey populations. Range expansion by the jumbo squid, *Disodocus gigas* may be a demonstration of this plasticity in response to changes in the marine environment (Field et al. 2007, Zeidberg & Robison 2007). Furthermore, *D. gigas*, an important prey item for top predators and a predator of lower trophic level organisms, appears to be central to the energy flow within the food chain of the pelagic ecosystem of the central Gulf of California (Rosas-Luis et al. 2008). A greater understanding of the role of cephalopods in the food chain both as predators and prey will enhance our understanding of how they may respond to environmental change (Rodhouse 2013).

Cephalopods, particularly squid, are known to be important prey items of many top-level predators such as marine mammals, sea birds and predatory fish throughout the world (e.g. Ruiz-Cooley et al. 2004, Cherel et al. 2009, Daneri et al. 2012, Monteiro et al. 2015a, also see Collins & Rodhouse 2006 and Rodhouse 2013) including subtropical waters surrounding the island of Tasmania, off southern Australia. For example, marine

mammals such as the Australian fur seal *Arctocephalus pusillus* (Gales & Pemberton 1994, Arnould et al. 2011), as well as long-finned pilot whales *Globicephala melas edwardii* (Walters 2005) and bottlenose dolphins *Tursiops truncatus* (Gales & Pemberton 1992) all consumed cephalopods as part of their diet. Furthermore, analysis of stomach contents from stranded sperm whales *Physeter macrocephalus* and from a single stranded pygmy sperm whale *Kogia breviceps* in Tasmanian waters suggested these marine mammals prefer a predominantly cephalopod diet (Evans & Hindell 2004a, Beasley et al. 2013). Similarly, the little penguin *Eudyptula minor* forages on squid (Gales and Pemberton 1990) as does the short-tailed shearwater *Puffinus tenuirostris* that breeds in Tasmania but undertakes long foraging trips to distant waters and consumes subantarctic squid as part of its diet (Weimerskirch & Cherel 1998). Likewise, the southern lanternshark *Etmopterus baxteri* and brown lanternshark *Etmopterus unicolor* in Tasmanian waters also consume cephalopods, particularly deepwater cephalopods, as evidenced from stomach content data (Hallet & Daley 2011).

While cephalopods are often important dietary components of many higher level predators, they can also be a higher order predator themselves. Cherel and Hobson (2005) showed that the colossal squid *Mesonychoteuthis hamiltoni* is a top level predator feeding on squid and fish while also being prey to apex predators such as sperm whales (Evans & Hindell 2004a) and sleeper sharks *Somniosus cf. microcephalus* (Cherel & Duhamel 2004). Squid are characterized as voracious but opportunistic predators with the ability to feed on prey of varying sizes and prey type including zooplankton, crustaceans, squid and fish (Boyle & Rodhouse 2005, Rodhouse 2013). Due to heavy feeding rates and a generalist feeding strategy for squid in conjunction with their plasticity in response to fisheries and environmental change, squid have the potential to highly impact functional relationships between prey and resource within the food web (Coll et al. 2013). It is therefore important to assess the cephalopod predator-prey relationship within the food chain. However, research on the diet of cephalopod species is limited, in part due to the prey of cephalopods being macerated by the beak and therefore hard to identify. Despite this difficulty, recent research by Pethybridge et al. (2012, 2013) highlighted the use of complementary techniques such as fatty acid analysis in conjunction with traditional dietary analysis to ascertain the trophic dynamics of *Nototodarus gouldi* and *Todarodes filippovae* respectively, caught in

Tasmanian waters. They confirmed a copepod – myctophid – squid food chain, similar to that identified in the Southern Ocean around South Georgia for *Martialia hyadesi* (Rodhouse et al. 1992) and around New Zealand, Macquarie and Heard Island for the onychoteuthid squid *Moroteuthis ingens* (Jackson et al. 1998, Phillips et al. 2002, 2003).

Much of what we know about the trophic dynamics of Southern Ocean cephalopods stems from dietary studies of higher predators than as a result of dedicated scientific surveys focused on cephalopods (Rodhouse 2013). Subsequently, the difficulty in capturing oceanic and deepwater species has prompted the use of a technique that infers composition, abundance, and distribution of cephalopods based on beaks identified from predator stomachs (Cherel et al. 2004, Ménard et al. 2013). This technique involves conducting stable isotope analysis on identified cephalopod beaks from predator stomachs that are used as biological samplers of cephalopods. As a result our understanding of the trophic ecology of cephalopods, especially deep-sea cephalopods, as well as the feeding ecology of the predator themselves has been furthered, including knowledge of new trophic structures and communities (Cherel & Hobson 2005, Cherel et al. 2009, Ruiz-Cooley et al. 2012, Xavier et al. 2015).

$\delta^{15}\text{N}$ values show a predictable stepwise enrichment with each increasing trophic level and have been successfully used to determine both the trophic level and existing relationship between predator and prey (Herman et al. 2005, Ruiz-Cooley et al. 2012). In contrast, $\delta^{13}\text{C}$ values vary little along the food chain and are used to signify the primary source of the food chain, making it an extremely useful indicator for migration patterns when the ratio of the consumer is compared to isotopically distinct geographical regions (Kurle & Worthy 2002).

The overall rationale of this study was to use stable isotope analysis to provide insights into the trophic relationship and feeding habits of a cephalopod community in waters surrounding Tasmania, Australia, north of the Subtropical Front. This project used only beaks from cephalopods caught predominantly by fishermen as by-catch at known times from known locations to help determine the trophodynamics of the cephalopod community in the subtropical waters of this region specifically, but also in comparison to other areas. Moreover, since different tissues have varying turnover rates (renewal time of tissue) a separate objective was to compare soft tissues (mantle and buccal

mass) with the hard tissue of the beak in various cephalopod species. Different tissues subsequently have different discrimination factors (the isotopic difference between the consumer and its food). In the absence of muscle tissue, the isotopic difference between the soft tissues (e.g. mantle) and hard tissue (beak) provides a correction factor for the beak when comparing beaks to predator tissues.

METHODS

Collection details

Cephalopod samples were collected opportunistically from waters around Tasmania, Australia in the ocean north of the Subtropical Front to examine the trophic ecology of species via isotopic analysis. Each fishing season squid were obtained incidentally from the commercial deepwater trawl fishing industry operating off southern and eastern Tasmania from 2001 to 2006. Similarly, samples of *Sepioteuthis australis*, *Nototodarus gouldi*, *Sepia apama* and *Octopus maorum* were obtained from other commercial fishermen operating in nearshore Tasmanian waters (Figure 2.1). Cephalopods from other regions used for trophic comparative purposes were obtained by trawling undertaken by research vessels. Specimens of *Slosarczykovia circumantarctica* were obtained from a research cruise on the vessel RV *Aurora australis* during June 2000 from waters around Macquarie Island (54°S, 159°E). Likewise, *Histioteuthis eltaninae* and *Galiteuthis glacialis* were collected on a cruise around Macquarie Island undertaken on the vessel RV *Aurora australis* using an International Young Gadoid Pelagic Trawl (IYGPT) during December 2000 and January 2001. Samples of *Moroteuthis ingens* from New Zealand Southern Plateau subantarctic waters (~48°S, 168°E) were acquired from a research cruise on the RV *Tangaroa*, equipped with a bottom trawl, during December 2000. *M. ingens* samples were also obtained from a research cruise on the RV *Aurora australis* during June 2000 from waters around Heard Island. (53°S, 73.3°E). In April 2006 a sample of *Sthenoteuthis oualaniensis* was obtained from a research cruise in northeast Australia, inshore off Mooloolaba, Queensland (26.7°S, 153.1°E) (Table 2.1). Upon capture cephalopods were either immediately frozen on board ship or packed fresh on ice until port. At port the frozen squid were stored in a -20°C freezer until further analysis while the fresh squid kept on ice were either frozen or immediately dissected. A subset of five squid species and one octopus species, used for isotopic comparison between tissues and beak, were all frozen prior to dissection.

Table 2.1 Capture details for all cephalopod species in this study, along with number (n), habitat and range in dorsal mantle length in mm (DML) of specimens for each species. SAW, subantarctic water; STW, subtropical water.

Family	Species	Capture Date	Location	Habitat	n	DML range (mm)
Sepia	<i>Sepia apama</i>	Dec 2002	West Coast Tasmania	STW, neritic	9	130-205
Loliginidae	<i>Sepioteuthis australis</i>	Jan 2007	East Coast Tasmania	STW, neritic	9	234-350
Enoplateuthidae	<i>Ancistrocheirus lesueuri</i>	Aug 2002	Tasmania	STW, pelagic	1	210
Octopoteuthidae	<i>Octopoteuthis</i> sp.	Nov 2000, Mar 2003, Feb/Apr 2004, 1 unknown	South Coast Tasmania	STW, benthopelagic	9	105-194
	<i>Taningia danae</i>	Dec 2001, Jan 2002, May 2002, Oct 2002	South Coast Tasmania	STW, pelagic	4	165-749
Onychoteuthidae	<i>Moroteuthis ingens</i>	Jan 2002, Dec 2003	South Coast Tasmania	STW, benthopelagic	9	430-563
		Dec 2000	NZ Southern Plateau	SAW, benthopelagic	29	235-461
		Feb 2000	Heard Island	SAW, benthopelagic	8	190-225
Lepidoteuthidae	<i>Pholidoteuthis boschmai</i>	Nov 2005	East Coast Tasmania	STW, pelagic	1	505
Histioteuthidae	<i>Histioteuthis atlantica</i>	April/May 2002, 1 unknown	South Coast Tasmania	STW, pelagic	15	47-290
	<i>Histioteuthis eltaninae</i>	Dec 2000/Jan 2001	Macquarie Is	SAW, pelagic	12	52-140
	<i>Histioteuthis macrohista</i>	Apr/May 2002, Feb/Mar 2004, Mar 2005	South Coast Tasmania	STW, pelagic	9	45-60
Brachioteuthidae	<i>Histioteuthis miranda</i>	May 2002	West Coast Victoria	STW, pelagic	11	240-265
	<i>Slosarczykovia circumantarctica</i>	June 2000	Macquarie Is	SAW, pelagic	5	143-197

Ommastrephidae	<i>Todarodes filippovae</i>		East Coast and South Coast Tasmania	STW, pelagic	53	285-538
	<i>Nototodarus gouldi</i>	Nov 2006	West Coast Tasmania	STW, pelagic	9	256-317
	<i>Ommastrephes bartrami</i>	unknown	Victoria	STW, pelagic	1	545
	<i>Sthenoteuthis oualaniensis</i>	Apr 2006	Mooloolaba, East Coast Australia	STW, pelagic	9	215-292
Chroteuthidae	<i>Chroteuthis veranyi</i>	Oct 2004, Mar 2005, 1 unknown	South Coast Tasmania	STW, pelagic	3	120-210
Mastigoteuthidae	<i>Idioteuthis cordiformis</i>	Oct 2003/2004	South Coast Tasmania	STW, benthopelagic	10	490-620
Cranchiidae	<i>Teuthowenia pellucida</i>	Feb/Mar 2004, Mar 2005, 1 unknown	South Coast Tasmania	STW, pelagic	6	111-150, 1 unknown
	<i>Galiteuthis glacialis</i>	Jan 2001	Macquarie Is	SAW, pelagic	9	165-391
Octopodidae	<i>Octopus maorum</i>	Dec 2006	South Coast Tasmania	STW, benthic	5	151-200

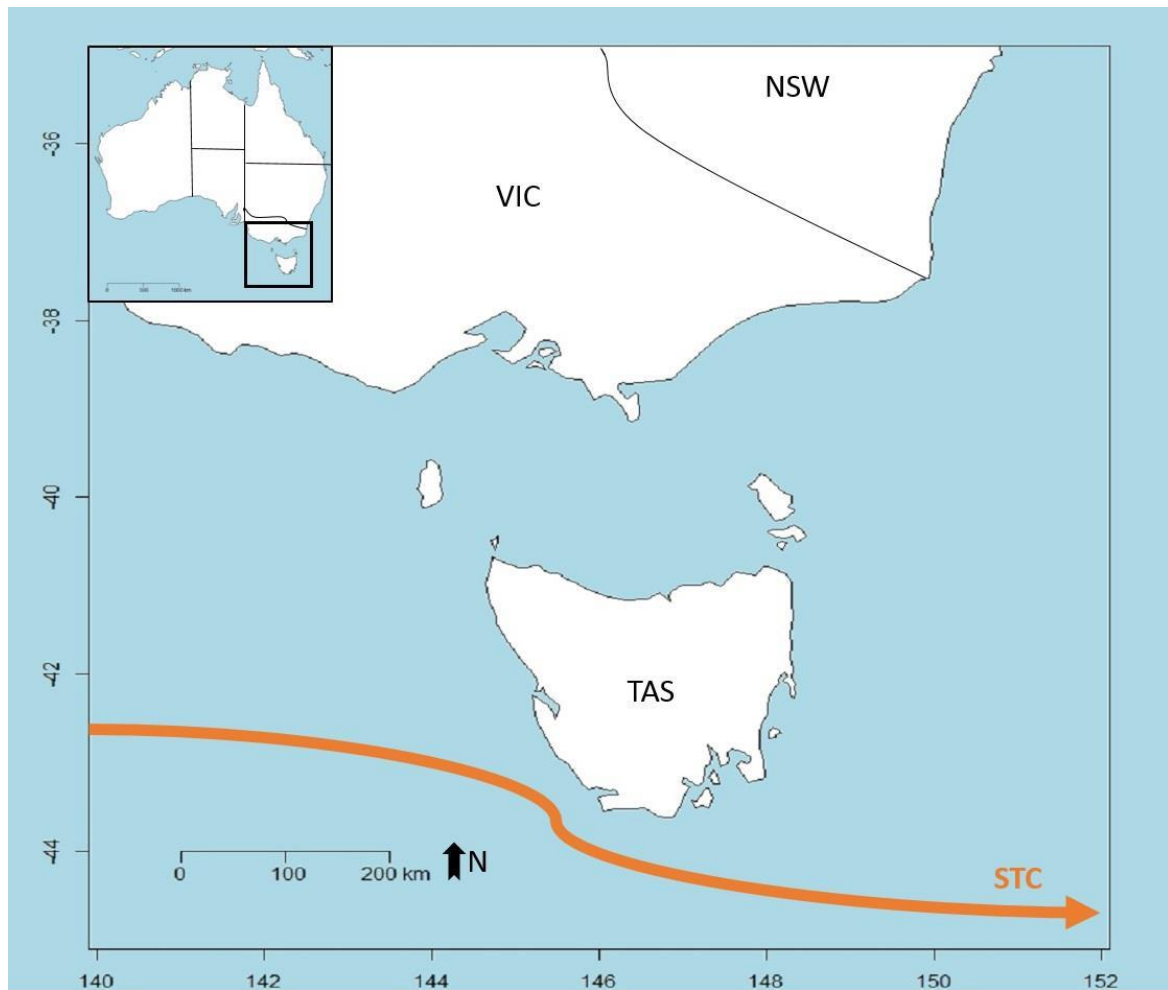


Figure 2.1. Map of southeastern Australia showing sampling regions around Tasmania and off Victoria. STC = Subtropical Convergence, TAS = Tasmania, VIC = Victoria, NSW = New South Wales.

Laboratory details

Once defrosted, cephalopods were identified to species level where possible. For each specimen the dorsal mantle length (DML, mm) and sex was recorded. Prior to isotopic analysis lower and upper beaks were removed and stored in 70% ethanol. Beaks were subsequently removed from ethanol and cleaned with distilled water. Lower rostral lengths (LRL) for squid were measured with digital calipers to the nearest millimeter (mm). A small piece of the tip of the wing in the direction of growth was then sampled from each beak and rinsed with distilled water. The tip of the wing was chosen as it represents the most recent growth phase of the beak and hence squid growth (Cherel &

Hobson 2005). Beaks were not delipidated (after Cherel & Hobson 2005). Isotopic analyses in tissues of five species of squid and one species of octopus were undertaken by comparing the mantle and buccal mass tissues with beaks from the same individuals (Hobson & Cherel 2006). Replicate individuals were analyzed for each species. Approximately 1 cm² pieces of mid-anterior mantle as well as small pieces of buccal mass were dissected, cleaned of any skin or mesentery and rinsed with distilled water. Samples were then frozen in preparation for isotopic analysis. Just prior to isotopic analysis, samples were freeze dried, ground to a small powder with a Wig-L-Bug and delipidated in cyclohexane. The ground mantle and buccal mass samples along with 3 ml of cyclohexane were left to stand overnight in small covered test tubes under a fume hood. After delipidating overnight, samples were then centrifuged and cyclohexane removed with a pipette. Samples were subsequently subjected to two more cyclohexane rinses. Between rinses the samples were allowed to stand for another 1 - 2 hours. Following delipidation samples were left to dry uncovered under a fume hood overnight. Relative abundance of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes was determined by a Finnigan Delta Plus Advantage stable isotope-ratio mass spectrometer at the University of Victoria, British Columbia, Canada. For isotopic analysis, beak wing tip samples were analyzed whole or cut in half for large specimens. For all isotopic analysis there was a 10 percent replication measurement for each isotopic sample run. The results of the isotopic analysis are presented in the usual δ notation relative to PDB belemnite for $\delta^{13}\text{C}$ and atmospheric N₂ (AIR) for $\delta^{15}\text{N}$. Replicate measurements of internal laboratory standards (DORM) indicated measurement errors of $\pm 0.1\text{‰}$ and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Ten percent replication within stable isotope runs revealed measurement errors of 0.2 ‰ for $\delta^{13}\text{C}$ and 0.3 ‰ for $\delta^{15}\text{N}$.

Statistical analyses

A two-way full factorial mixed model analysis of variance (ANOVA) with tissue type and species as fixed factors and individual nested within species as a random term was used to determine if the isotopic discrimination between the mantle, buccal mass and beaks of different species was consistently different from one another. The trophic structure of the cephalopod community from Tasmanian waters was analyzed using ANOVA. While every effort was made to have similar samples sizes this was not always possible.

Species with less than three individuals were not used in the analysis. However, data was screened to meet assumptions of ANOVA. As much as possible similar sized animals were chosen for replication within a species. Closely related histioteuthid species were also examined to determine any resource partitioning within the family.

In addition, ANOVAs were also used to establish if there was any seasonal, annual, location or sex differences in the average $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of beaks extracted from *T. filippovae* caught off the south and east coast of Tasmania between 2005 and 2006. For all additional species where there was a size range, correlation analysis was calculated between $\delta^{15}\text{N}$ values from beaks and DML of individuals. Since $\delta^{15}\text{N}$ values are used as an indicator of trophic level it was expected that any progressive increase in $\delta^{15}\text{N}$ values with DML would signify a dietary shift to higher-trophic level prey.

Similarly, a multivariate analysis of variance (MANOVA) with sex and size as the factors of interest was used to examine differences simultaneously in the average $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of beaks from *M. ingens* caught in New Zealand waters. Due to low numbers in size and sex for the Tasmanian and Heard Island samples, only New Zealand data was used in this analysis. Furthermore, to determine if location influenced the stable isotopic signature of the beaks of *M. ingens*, MANOVA was used to compare the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaks concurrently. Since a full size range of individuals was not available from each location, separate MANOVAs for large individuals caught from Tasmanian and New Zealand waters and smaller individuals from New Zealand and Heard Island were computed.

All data was log-transformed, however where assumptions of a parametric test (normality) could not be met by transformation of the data, a non-parametric test was used. When a significant interaction or main effect was obtained in the full factorial models, a Tukey's honestly significant post-hoc test was computed to determine where the significant group differences were occurring.

RESULTS

A total of 15 species of squid, one species of octopus and one species of cuttlefish from Tasmanian waters were analyzed to compare their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (see Table 2.2). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *S. australis* were obtained from muscle samples and corrected using the difference between three beaks and the mantle of the

Table 2.2. Dorsal mantle length (DML), lower rostral length (LRL), lower beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and C:N mass ratio values of the squid species living in Southern Ocean waters around Tasmania. *Histioteuthis eltaninae*, *Slosarczykovia circumantarctica* and *Galiteuthis glacialis* from Macquarie Island and *Sthenoteuthis oualaniensis* from eastern Australia were used as comparisons. All values are means \pm SD. ND = no data. Numbers in brackets indicate where numbers are less than the total n.

Family	Species	n	DML (mm)	LRL (mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio
Sepia	<i>Sepia apama</i>	9	114 \pm 7	3.1 \pm 1.3	-17.2 \pm 0.3	8.3 \pm 0.3	3.2 \pm 0.2
Loliginidae	<i>Sepioteuthis australis</i>	9	285 \pm 38	ND	-17.2 \pm 0.3	11.7 \pm 0.2	2.9 \pm 0.0
Enoploteuthidae	<i>Ancistrocheirus lesueuri</i>	1	210	6.5	-17.0	8.2	3.0
Octopoteuthidae	<i>Octopoteuthis</i> sp.	9	140 \pm 35 (7)	7.7 \pm 1.3 (6)	-18.3 \pm 0.4	9.3 \pm 0.7	3.1 \pm 0.2
	<i>Taningia danae</i>	3	212 \pm 47	9.3 \pm 0.6	-17.6 \pm 0.4	8.8 \pm 0.5	3.6 \pm 0.2
	<i>Taningia danae</i>	1	749	ND	-18.2	8.5	4.0
Onychoteuthidae	<i>Moroteuthis ingens</i>	9	480 \pm 52	11.5 \pm 0.4	-18.6 \pm 0.6	6.7 \pm 1.1	3.3 \pm 0.2
Lepidoteuthidae	<i>Pholidoteuthis boschmai</i>	1	505	9.3	-18.2	9.6	3.8
Histioteuthidae	<i>Histioteuthis atlantica</i>	11	162 \pm 20	5.7 \pm 0.4 (10)	-17.7 \pm 0.4	10.1 \pm 0.5	3.4 \pm 0.2
	<i>Histioteuthis eltaninae</i>	9	113 \pm 12	3.5 \pm 0.2	-21.1 \pm 0.5	6.5 \pm 0.6	3.5 \pm 0.2
	<i>Histioteuthis macrohista</i>	9	51 \pm 6	3.5 \pm 0.3 (8)	-17.8 \pm 0.4	8.4 \pm 0.9	2.3 \pm 1
	<i>Histioteuthis miranda</i>	11	248 \pm 9	6.7 \pm 0.3	-18.1 \pm 0.1	11.8 \pm 0.8	3.3 \pm 0.5
Brachioteuthidae	<i>Slosarczykovia circumantarctica</i>	5	164 \pm 20	3.6 \pm 0.5	-22.7 \pm 2.1	5.7 \pm 1.6	2.9 \pm 0.9
Ommastrephidae	<i>Todarodes filippovae</i>	10	425 \pm 52	10.9 \pm 1.5	-17.5 \pm 0.2	8.3 \pm 1.1	3.6 \pm 0.3
	<i>Nototodarus gouldi</i>	9	274 \pm 18	8.0 \pm 0.7	-17.4 \pm 0.4	9.6 \pm 0.6	3.4 \pm 0.1
	<i>Ommastrephes bartrami</i>	1	545	13.8	-17.0	7.1	3.4
	<i>Sthenoteuthis oualaniensis</i>	9	244 \pm 24	ND	-17.8 \pm 0.3	6.3 \pm 0.3	3.4 \pm 0.1
Chiroteuthidae	<i>Chiroteuthis veranyi</i>	3	153 \pm 49	7.6 \pm 0.7	-17.9 \pm 0.3	9.0 \pm 0.2	2.5 \pm 1.1
Mastigoteuthidae	<i>Idioteuthis cordiformis</i>	10	540 \pm 42	13.9 \pm 0.7	-16.7 \pm 0.3	12.0 \pm 0.5	3.4 \pm 0.1
Cranchiidae	<i>Teuthowenia pellucida</i>	6	134 \pm 14 (5)	3.7 \pm 0.8	-17.9 \pm 0.4	7.0 \pm 1.9	2.3 \pm 0.9
	<i>Galiteuthis glacialis</i>	6	346 \pm 32	4.0 \pm 0.3	-20.8 \pm 0.3	6.4 \pm 1.1	3.3 \pm 0.8
Octopodidae	<i>Octopus maorum</i>	5	169 \pm 20	ND	-17.2 \pm 0.8	10.6 \pm 0.4	3.2 \pm 0.1

same individuals so as to be comparable to all other beaks. A correction value of -1.0 ‰ and $+4.3$ ‰ was used for correcting the mantle values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively. Similarly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaks for *Octopoteuthis* sp. were derived from correcting mantle/fin tissue values based on correction values of -0.7 ‰ and $+3.8$ ‰ respectively (see tissue comparison results below). Additionally, the isotopic values on another five species (*M. ingens*, *S. oualaniensis*, *S. circumantarctica*, *G. glacialis*, *H. eltaninae*) from different locations for comparative purposes were also determined.

Tissue comparisons

The isotopic signatures of beaks, buccal masses and mantles were dependent on species by site (mantle, buccal mass or beak) interaction for both $\delta^{13}\text{C}$ ($F_{10,90} = 27.1$, $p < 0.0001$) and $\delta^{15}\text{N}$ ($F_{10,90} = 10.5$, $p < 0.0001$). Overall, beaks had the highest $\delta^{13}\text{C}$ values (-17.8 ± 0.8 ‰) whereas the buccal masses had the lowest values (-18.8 ± 1.0 ‰). For all species, the $\delta^{13}\text{C}$ values of beaks were on average 1.0 ‰ higher than buccal masses, except for *S. oualaniensis* where the beaks were similar to the buccal mass. Moreover, the $\delta^{13}\text{C}$ values of beaks were on average 0.7 ‰ higher than mantles. The mantles in general were 0.3 ‰ more enriched in ^{13}C than the buccal masses except for *H. macrohista*, which appeared to exhibit a marginal impoverishment between the tissues in comparison to other species.

Overall the average $\delta^{15}\text{N}$ values of the mantle from all species (11.9 ± 1.5 ‰) were the highest. There was an average discrimination factor of 0.6 ‰ and 3.8 ‰ between the mantle and buccal mass, and mantle and beak respectively. Similarly, the $\delta^{15}\text{N}$ value of the buccal mass was on average 3.2 ‰ higher than the beak. The only exception to the overall pattern was for *Histioteuthis macrohista* where the buccal mass had similar or marginally higher $\delta^{15}\text{N}$ values compared to the mantle (Figure 2.2).

Ontogenetic differences

There was no evidence of season, year, location or sex differences found in the beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *T. filippovae* collected in Tasmanian waters. Consequently, all *T. filippovae* individuals were combined in a correlation analysis to determine if $\delta^{15}\text{N}$ values progressively increased with size. The analysis revealed a moderate positive association between $\delta^{15}\text{N}$ values and DML ($r = 0.57$, $n = 53$, $p < 0.0001$). Correlation analysis also revealed that the $\delta^{15}\text{N}$ values of beaks were related to DML in *H. eltaninae*

($r = 0.73$, $n = 12$, $p < 0.01$), *Histioteuthis atlantica* (Spearman's $\rho = 0.84$, $n = 15$, $p < 0.0001$) and *G. glacialis* ($r = 0.96$, $n = 8$, $p < 0.0001$). All of the species revealed that the beaks became more enriched in ^{15}N as DML increased.

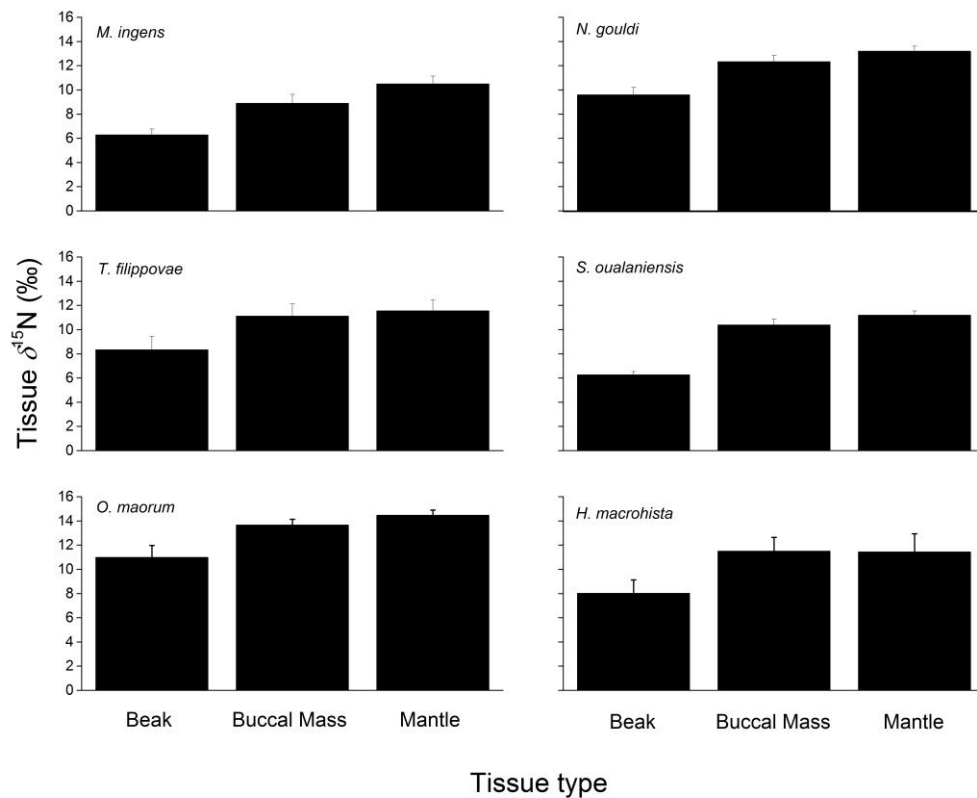


Figure 2.2. Differences in mean $\delta^{15}\text{N}$ values for lower beak, buccal mass and mantle of *Moroteuthis ingens*, *Nototodarus gouldi*, *Todarodes filippovae*, *Sthenoteuthis oualaniensis*, *Octopus maorum* and *Histioteuthis macrohista*.

Beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from individuals of *M. ingens* captured in New Zealand waters showed evidence of a sex \times size interaction when compared simultaneously in multivariate space (Wilks lambda $_{2,25} = 4.6$, $p < 0.05$). However, this must be interpreted with caution due to an influential outlier in the $\delta^{13}\text{C}$ value of a small female. Subsequently, when this observation was removed from the model, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values were shown to be dependent on size (Wilks lambda $_{2,24} = 8.2$, $p < 0.01$).

and sex (Wilks lambda $_{2,24} = 3.6$, $p < 0.05$) in multivariate space (Wilks lambda $_{2,24} = 8.4$, $p < 0.05$). However, in univariate space $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values were only dependent on size ($F_{1,25} = 7.9$, $p = 0.01$; $F_{1,25} = 16.7$, $p < 0.0001$ respectively). Beaks from larger individuals were on average more enriched in ^{13}C by 0.8 ‰. Similarly, larger individuals of *M. ingens* (DML = 385 ± 44 mm) were more enriched in ^{15}N than smaller individuals (DML = 258 ± 16 mm) by an overall increase of 1.7 ‰ (Figure 2.3). Females were more enriched in ^{13}C than males by 0.5 ‰ but males (6.5 ± 1.5 ‰) had higher $\delta^{15}\text{N}$ values than females (5.7 ± 1.0 ‰) (Figure 2.3). On average females (DML 380 ± 79 mm) were larger than males (DML = 318 ± 49 mm).

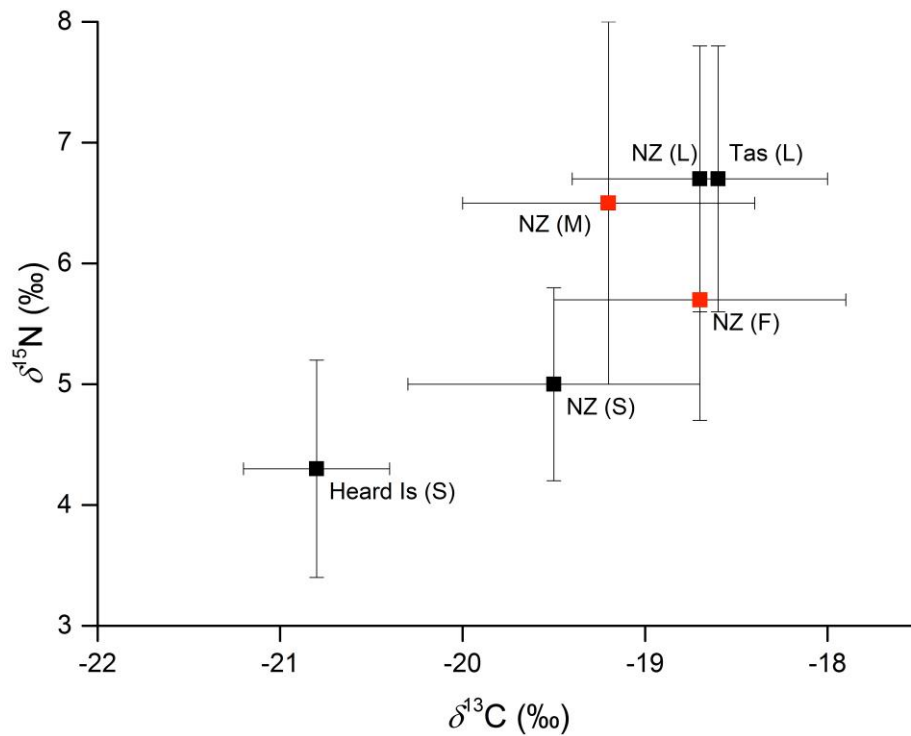


Figure 2.3. Lower beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Moroteuthis ingens* of different sizes and from different localities (Tasmania, New Zealand and Heard Island). Abbreviations: NZ, New Zealand; Tas, Tasmania; S, small; L, large; M, males; F, females. Values are mean \pm SD. Red squares indicate the New Zealand male and female individuals.

The concurrent analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaks from larger individuals of *M. ingens* from New Zealand and Tasmania were shown not to be dependent on location.

Conversely, location did have an effect on the $\delta^{13}\text{C}$ values of beaks from smaller individuals caught around Heard Island and New Zealand (Wilks lambda $_{2,15} = 3.88$, $p < 0.05$). ANOVA revealed that Heard Island individuals were more depleted in ^{13}C ($-20.8 \pm 0.4 \text{ ‰}$) than New Zealand individuals ($-19.5 \pm 0.8 \text{ ‰}$) (Figure 2.3).

Trophic structure of Tasmanian cephalopod community

The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaks from individuals of the Tasmanian cephalopod community as well as a comparison with squid from other locations were dependent on species ($F_{17,124} = 28.04$, $p < 0.0001$, $F_{17,124} = 33.77$, $p < 0.0001$ respectively). Although the ANOVAs for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were rerun without outliers, there was no overall difference in the results and therefore both analyses were computed inclusive of outliers. The range in average $\delta^{13}\text{C}$ values for the Tasmanian cephalopod community and comparison squid showed evidence of a gradual enrichment from -22.7 to -16.7 ‰ . However, when examining only the Tasmanian cephalopod community the range was much more restricted ($-18.6 \pm 0.6 \text{ ‰}$ *M. ingens* to $-16.7 \pm 0.3 \text{ ‰}$ *I. cordiformis*). The beaks of the Tasmanian community as well as the comparison squid separated into three distinct groups based on $\delta^{13}\text{C}$ values according to the post-hoc Tukey's tests. *S. circumantarctica* which was the most depleted in carbon (-22.7 ‰) separated into its own group as did *H. eltaninae* and *G. glacialis* (-21.1 ± 0.5 and $-20.8 \pm 0.3 \text{ ‰}$, respectively) which are all from Macquarie Island. The third significantly different group, which was more enriched in ^{13}C than the other two groups, contained all other cephalopods including all species from the Tasmanian community. *S. oualaniensis* from waters off eastern Australia and *Histioteuthis miranda* from the west coast of Victoria were both within the same restricted $\delta^{13}\text{C}$ range as the Tasmanian community (see Figure 2.4).

Tukey's post-hoc tests revealed that beak $\delta^{15}\text{N}$ values separated cephalopods into five groups with graduating enrichment (Figure 2.5). It is worth noting that the least enriched ^{15}N group contained all of the comparison species (*S. circumantarctica*, *G. glacialis*, *H. eltaninae* and *S. oualaniensis*) but only included *M. ingens* and *Teuthowenia pellucida* from the Tasmanian community. Of the cephalopods most enriched in ^{15}N , *Idioteuthis cordiformis*, *H. miranda* and *S. australis* were significantly different from any of the other groups. The overall range in mean $\delta^{15}\text{N}$ values for the Tasmanian

community was 6.7 ± 1.1 ‰ (*M. ingens*) to 12 ± 0.5 ‰ (*I. cordiformis*) with a difference of 5.3 ‰ or 1.5 - 2 trophic levels (Figure 2.5).

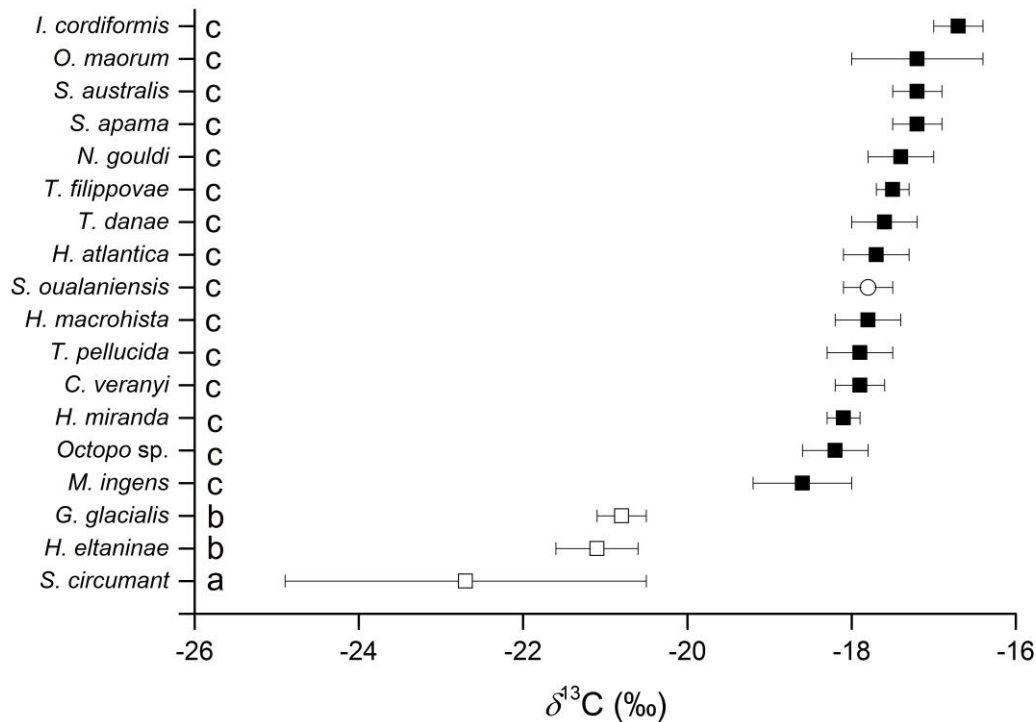


Figure 2.4. Beak $\delta^{13}\text{C}$ values for cephalopods captured in the subtropical waters around Tasmania. Open square symbol refers to comparison species from Macquarie Island, *Galiteuthis glacialis*, *Histioteuthis eltaninae* and *Slosarczykovia circumantarctica*. Open circle symbol refers to *Sthenoteuthis oualaniensis* from eastern Australia. Lower case letters refer to groups according Tukey's post-hoc test. *S. circumant*, *S. circumantarctica*; *Octopo* sp., *Octopoteuthis* sp. Values are means \pm SD.

An analysis of the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaks from three histioteuthid species from the Tasmanian community revealed that resource partitioning was dependent on species (Wilks lambda $_{4,54} = 16.84$, $p < 0.0001$). Univariate analysis showed no separation based on beak $\delta^{13}\text{C}$ values but histioteuthid beaks separated based on $\delta^{15}\text{N}$ values ($F_{2,28} = 46.81$, $p < 0.0001$). Post-hoc Tukey's test revealed that all species were distinctly different from one another. *H. miranda* had the highest $\delta^{15}\text{N}$

value (11.7 ± 0.8 ‰), followed by *H. atlantica* (10.1 ± 0.5 ‰) and *H. macrohista* with the lowest $\delta^{15}\text{N}$ value (8.3 ± 0.9 ‰) (Figure 2.6).

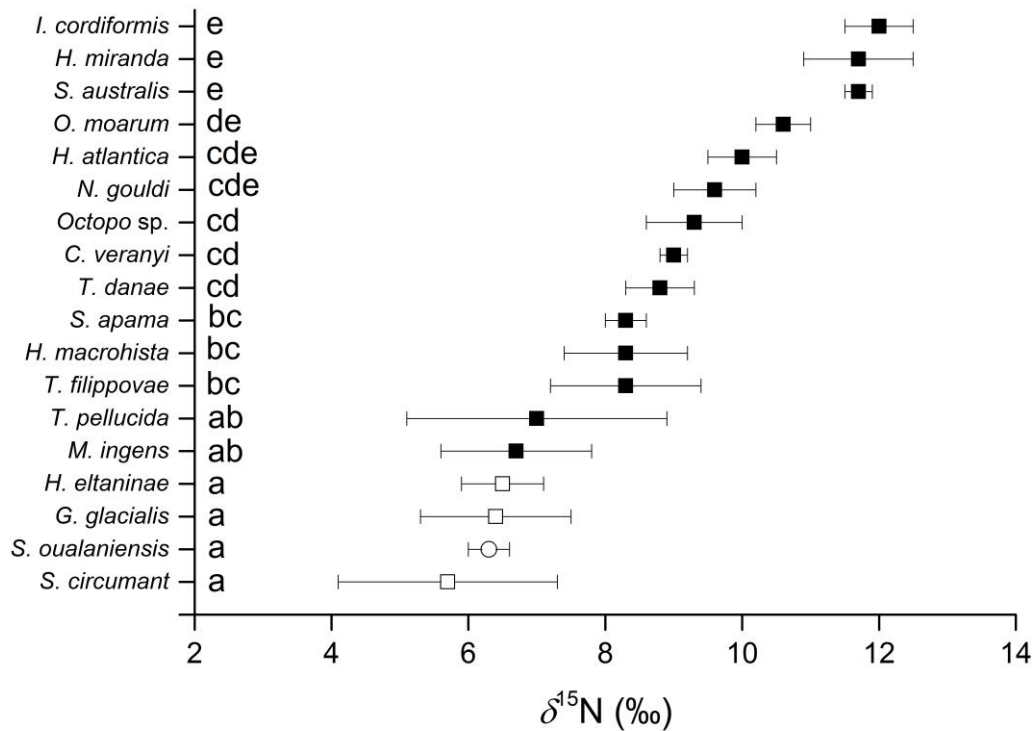


Figure 2.5. Beak $\delta^{15}\text{N}$ values for cephalopods captured in the subtropical waters around Tasmania. Open square symbol refers to comparison species from Macquarie Island, *Galiteuthis glacialis*, *Histioteuthis eltaninae* and *Slosarczykovia circumantarctica*. Open circle symbol refers to *Sthenoteuthis. oualaniensis* from eastern Australia. Lower case letters refer to groups according Tukey's post-hoc test. *S. circumant*, *S. circumantarctica*; *Octopo* sp., *Octopoteuthis* sp. Values are means \pm SD.

DISCUSSION

Tissue comparisons

As anticipated, this study indicated small variations in ^{13}C between tissues of the same species of cephalopod compared to ^{15}N . Overall, there was a 1 ‰ difference in $\delta^{13}\text{C}$ values between the more enriched ^{13}C buccal mass compared to the ^{13}C depleted beaks

which is identical to that found for *D. gigas* (Ruiz-Cooley et al. 2006). However, it is less than the 1.4 ‰ found for another squid species *Psychroteuthis glacialis* when the whole beak as opposed to the wing tip was compared to the buccal mass (Cherel & Hobson 2005). The overall difference in $\delta^{13}\text{C}$ values between the mantle and beak of all cephalopod species was slightly less (0.7 ‰) than for the buccal mass but in accordance to that found for *T. filippovae* (0.6 ‰) (Cherel et al. 2009). Moreover, the $\delta^{13}\text{C}$ values are similar to the discrimination factor between soft tissues and the beak obtained for cuttlefish *Sepia officinalis* reared in captivity and fed a known diet (Hobson & Cherel 2006). These $\delta^{13}\text{C}$ differences are also within the ranges found when comparing multiple tissues from the same individuals of other cephalopods (Hobson & Cherel 2006, Cherel et al. 2009b). This suggests that using a correction factor for $\delta^{13}\text{C}$ values of cephalopod beaks when comparing between beaks of different species of cephalopods or between beaks and potential prey is not essential.

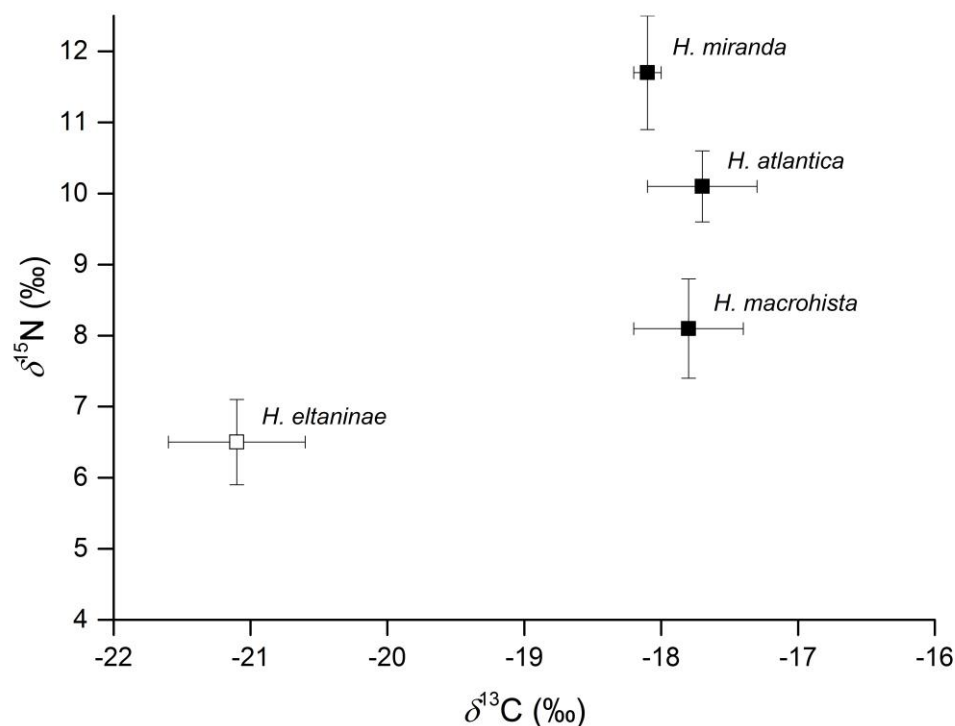


Figure 2.6 Beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 3 histioteuthid species *Histioteuthis miranda*, *Histioteuthis atlantica* and *Histioteuthis macrohista* from Tasmanian waters, *Histioteuthis eltaninae* is a comparison species from Macquarie Island. Values are means \pm SD.

This study also confirmed that across a variety of cephalopod species the soft tissues of the mantle and buccal mass are more enriched in ^{15}N than the hard chitinized beaks. Beaks contain a large amount of chitin (Hunt & Nixon 1981) which when compared to soft tissues and food is depleted in ^{15}N (DeNiro & Epstein 1978) resulting in a lower $\delta^{15}\text{N}$ value for the beaks. The overall difference between mantle and beak wing (3.8 ‰) for all our species corroborates the documented difference observed between the same tissues for *T. filippovae* (3.5‰) caught in the southwestern Indian Ocean (Cherel et al. 2009b). Similarly, these results support the 3 – 4 ‰ difference between the ^{15}N enriched arm muscle and ^{15}N depleted whole beaks found in *P. glacialis* (Cherel & Hobson 2005) and the buccal mass and whole wing of *D. gigas* (Ruiz-Cooley et al. 2006). Similarly, Hobson and Cherel (2006) found that soft tissues of *Todaropsis eblanae*, *Illex coindetti*, *Loligo vulgaris* and *S. officinalis* were substantially enriched in ^{15}N relative to whole beaks (4.8 ‰). This highlights the need to use correction factors when using cephalopod beaks in assessing the trophic dynamics or organic pathways between both cephalopod predator and prey.

Since dietary isotopic shift appeared to be similar between the cephalopod species in this study, it would suggest that when comparing beaks from different species they would not require correction. However, this will depend on the chitin to protein ratio of the beaks (Cherel et al. 2009b). Beaks vary in the chitin to protein ratio considerably, with undarkened wings of beaks having a greater chitin to protein ratio than darkened wings, which is associated with squid size and maturity (Miserez et al. 2007). Chitin is depleted in ^{15}N relative to the predator's prey and also has a C:N mass ratio that is higher than protein (Schimmelmann & DeNiro 1986). Subsequently, a beak with a high chitin to protein ratio is likely to have a lower $\delta^{15}\text{N}$ value and a higher C:N mass ratio relative to a beak with lower amounts of chitin (Cherel et al. 2009b). This highlights the importance of examining the C:N mass ratio when comparing cephalopod beak $\delta^{15}\text{N}$ values. A few species in this study had particularly low C:N ratios (i.e. *Teuthowenia pellucida*, *H. macrohista*, *Chroteuthis veranyi*) and therefore need to be compared carefully (see Table 2.2).

Using isotopic analysis on hard chitinous cephalopod beaks from predator stomachs to describe cephalopod trophic community structure has been useful in analyzing the trophic structure of poorly known cephalopod communities in various locations (Cherel

et al. 2009a, Xavier et al. 2014, Xavier et al. 2015, Negri et al. 2016, Seco et al. 2016). Ménard et al. (2013) were able to describe the importance of pelagic cephalopods in the western Indian Ocean based on the isotopic comparison of beaks found in the stomachs of a number of predatory fish and a seabird. Similarly, a top predator, the wandering albatross *Diomedea exulans* was used as a biological sampler to reconstruct cephalopod assemblages and examine trophic relationships in the south Atlantic and Indian sectors of the Southern Ocean (Guerreiro et al. 2015). A deepwater cephalopod assemblage in the northeast Atlantic was also documented using sperm whales as samplers (Cherel et al. 2009a).

Ontogenetic changes

Body size is a key factor in the structuring of marine food webs and consequently an important component in funneling the flow of energy up the food chain, from the smallest to the largest organism (Parry 2008). As expected, the increase in $\delta^{15}\text{N}$ values with DML in the subset of five species of cephalopods analyzed in this study supports the theory of ontogenetic dietary shifts. Trophic analysis reveals for many fish and squid that dietary habits are size-structured (Revill et al. 2009). Cephalopods occupy a number of trophic levels throughout their lifespan. They prey on species lower in the food chain in their juvenile stages such as crustaceans. As they grow and mature, they shift to a primarily piscivorous diet (Rodhouse & Nigmatullin 1996). Uchikowa and Kidokoro (2014) found clear shifts with ontogeny in juvenile squid of *Todarodes pacificus*. The smaller juveniles preyed predominantly on crustaceans as opposed to the larger juveniles that shifted toward a predominantly fish-based diet. Stomach content analysis and fatty acid analysis confirmed a copepod – myctophid – squid food chain for *M. ingens* (Jackson et al. 1998, Phillips et al. 2003) and *T. filippovae* (Pethybridge et al. 2013) in subtropical and subantarctic waters.

The increasing $\delta^{15}\text{N}$ values of squid and other marine organisms with increasing body size is considered to be a product of the accumulation of heavier isotopes resulting from feeding on larger bodied prey at higher trophic levels (Kurle & Worthy 2001, Kurle et al. 2011). For *T. filippovae* in this study, the variability in isotopic values of $\delta^{15}\text{N}$ was accounted for by size-related differences as opposed to sex, geographical or seasonal factors. This shift in diet resulting from size changes or ontogeny using isotopic analysis has been broadly reported for a number of squid species. Hunsicker et al. (2010) found

that adult *Berryteuthis magister* increased by approximately one trophic level compared to the juvenile life stages. A comparable difference of at least one trophic level (around 3-4 ‰) across the lifespan was also documented for other oceanic squid such as *Kondakovia longimana* (Cherel & Hobson 2005) and *T. filippovae* (Cherel et al. 2009b). However, only half a trophic level was observed between large and smaller-sized *M. ingens* in this study (1.6 ‰) and also for *M. ingens* from Crozet and Kerguelen Islands (1.9 ‰) (Cherel & Hobson 2005). These results however may have possibly been due to a lack of specimens analysed at the lower end of the size spectrum. These positive correlations in $\delta^{15}\text{N}$ values with DML probably correspond to a diet of crustaceans by smaller squid to predominantly mesopelagic fishes by the larger squid (Philips et al. 2003, Cherel & Hobson 2005). Conversely, a greater shift in $\delta^{15}\text{N}$ values was reported in the transition from juvenile (6 ‰) to adult *Ommastrephes bartrami* (13 ‰) (Parry 2008). However, *O. bartrami* also reached a trophic plateau with size. This may be an effect of physical constraints on the squid or a lack of suitable prey items at higher trophic levels (Parry 2008). Ruiz-Cooley et al. (2010) found that based on isotopic analysis of the gladius of *D. gigas*, a smaller $\delta^{15}\text{N}$ difference (2.2 ‰) was found over the lifespan of the largest squid analyzed. A similar value was found when analyzing the gladius of *D. gigas* caught off Peru which was found to initially increase in $\delta^{15}\text{N}$ with size by 2.2 ‰ in the early part of its life, but then drop 4.6 ‰ near the latter part of its life, suggesting a late diet change from fish to euphausiids (Lorrain et al. 2011). This would tend to contradict the common findings of a systematic increase in $\delta^{15}\text{N}$ and hence trophic level with age, but may reflect the plasticity of this species and highlight opportunistic feeding.

In contrast to the pattern found for $\delta^{15}\text{N}$ values among different species, only a small but significant difference in $\delta^{13}\text{C}$ values (0.8 ‰) was found for larger compared to smaller sized *M. ingens*. Since benthic organisms are more enriched in ^{13}C than pelagic organisms, this may reflect an ontogenetic descent to deeper waters by females with maturation. *M. ingens* is sexually dimorphic with females being larger (Jackson 1997). This difference in $\delta^{13}\text{C}$ values may be correlated with an ontogenetic change in diet with growth, from crustaceans to mesopelagic prey, as evidenced by the change in $\delta^{15}\text{N}$ values. However, the males had a small increase in $\delta^{15}\text{N}$ values over the females (0.8 ‰). Since females cease to eat as they develop their huge egg mass, it would be

interesting to see if there is a $\delta^{15}\text{N}$ plateau. The differences were small and may not be biologically significant since similar differences can be found with isotopic discrimination within individuals. Furthermore, Cherel and Hobson (2005) found no significant difference between the $\delta^{13}\text{C}$ values of large and medium sized individuals of *M. ingens* from the Crozet and Kerguelen Islands. Future research may be able to shed more light on this.

Community structure

The Macquarie Island squids, *H. eltaninae* as well as *S. circumantarctica* and *G. glacialis* were used for $\delta^{13}\text{C}$ comparisons with cephalopods caught in Tasmanian waters. The $\delta^{13}\text{C}$ value of *S. circumantarctica* was significantly different from all other cephalopods, as was *H. eltaninae* and *G. glacialis*, which grouped together. The Tasmanian cephalopods showed a gradual increase in $\delta^{13}\text{C}$ values from *M. ingens* (-18.6‰) to *I. cordiformis* (-16.7‰) that was the most enriched in ^{13}C (Figure 2.4). The differences in $\delta^{13}\text{C}$ values found between the Macquarie Island and Tasmanian cephalopods corresponded to the higher $\delta^{13}\text{C}$ values typically found in warm subtropical waters at lower latitudes compared to the lower $\delta^{13}\text{C}$ values documented for colder Antarctic waters at higher latitudes (Rau et al. 1982, Trull & Amand 2001). This geographical gradient in $\delta^{13}\text{C}$ values is based on plankton food bases (phytoplankton and particulate organic matter, POM), which is in turn reflected in the $\delta^{13}\text{C}$ values of higher trophic organisms (Veit-Kohler et al. 2013) and has been observed for a number of marine organisms such as zooplankton (Schmidt et al. 2003), leopard seals (Hall-Aspland et al. 2005), penguins (Cherel & Hobson 2007) and other seabirds (Quillfeldt et al. 2010). The higher $\delta^{13}\text{C}$ values of cephalopods from Tasmanian waters encompassed a relatively narrow range (Figure 2.4). The small $\delta^{13}\text{C}$ gradient in this community may be interpreted in terms of benthic versus pelagic and nearshore versus offshore distribution, with benthic and nearshore/neritic being the most enriched in ^{13}C (Kurle et al. 2011). *I. cordiformis*, the most ^{13}C enriched is benthopelagic, followed by *Octopus maorum*, which is nearshore and benthic. Then there is a graduation from nearshore/neritic (*S. australis* and *Sepia apama*) to offshore oceanic and deepwater species. However, the deepwater squid *M. ingens*, suggested to have a benthopelagic lifestyle (Jackson 1993), had the lowest $\delta^{13}\text{C}$ values. This is likely due to their consumption of pelagic prey (myctophids) as the consumer retains the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

values of their assimilated prey. Furthermore, $\delta^{13}\text{C}$ values were not related to the size of individuals within the community, as there were large specimens at each end of the continuum. In contrast, size was related to $\delta^{13}\text{C}$ values in a deep-sea cephalopod assemblage in the northeast Atlantic, signifying the large squid *Taningia danae*, and *Lepidoteuthis grimaldi*, with more enriched carbon values, lived in the bathyal. This relationship also suggests that some species adopt a more demersal lifestyle as they age (Cherel et al. 2009a).

The $\delta^{15}\text{N}$ values of the community of cephalopods living in waters around Tasmania spanned almost two distinct trophic levels. There was a graduating continuum spanning from 6.7 ± 1.1 ‰ (*M. ingens*) to 12.0 ± 0.5 ‰ (*I. cordiformis*). This span in trophic levels is similar to other cephalopod communities inferred from isotopic analysis of beaks from predators. The diet of stranded sperm whales from the north Atlantic revealed a $\delta^{15}\text{N}$ continuum corresponding to approximately 1.5 trophic levels for deep-sea cephalopods (Cherel et al. 2009a). Similarly, the trophic relationship of the cephalopod assemblage estimated from the isotopic analysis of beaks from top predatory fish and seabirds feeding in the slope waters around the Kerguelen Islands showed that coexisting cephalopods fed along a continuum of two whole trophic levels (Cherel et al. 2011). The large range in trophic levels exhibited by cephalopods from the Kerguelen community was potentially higher than that reported for other coexisting closely related marine organisms (Cherel & Hobson 2005). This is consistent with cephalopods occupying a wide range of trophic levels and underscores their generalist feeding strategy. Furthermore, a near continuous gradient of $\delta^{15}\text{N}$ values or trophic levels points to an unstructured community of cephalopods feeding on a mixed diet sourced from a variety of trophic levels (Soares et al. 2014). Cephalopods are able to exploit resources across the entire food web. It is suggested that in ecosystems where the trophic width is broad the ecological features of the cephalopods vary. In contrast, in regions where the trophic width is narrow, the species seem to be more ecologically similar (Navarro et al. 2013).

The gradual enrichment in ^{15}N spanning almost two trophic levels for the Tasmanian cephalopod community strongly suggests, as has been suggested for subantarctic cephalopods, that their diet spans a continuum of lower trophic level crustacean-feeders or secondary consumers to higher trophic level fish-feeders or quaternary

consumers (Cherel & Hobson 2005). *M. ingens*, which had low $\delta^{15}\text{N}$ values in this study, is known to feed on euphausiids and mesopelagic fishes in the Southern Ocean (Jackson et al. 1998, Phillips et al. 2003). Based on a comparison between $\delta^{15}\text{N}$ values of species in this cephalopod community, *M. ingens* was grouped with *T. filippovae*. Similarly, *T. filippovae* then overlapped with *Nototodarus gouldi* in the next group on the $\delta^{15}\text{N}$ continuum, both of which have been documented as having a diet dominated by mesopelagic fishes but supplemented by squid and crustaceans in the Tasmanian region (Pethybridge et al. 2012). *N. gouldi* overlapped with *O. maorum*, which grouped with cephalopods with the highest $\delta^{15}\text{N}$ values, and has been found to feed on crustaceans and fish in Tasmanian waters (Grubert et al. 1999) (Figure 2.5).

M. ingens had the lowest $\delta^{15}\text{N}$ values in the cephalopod community with similar corrected $\delta^{15}\text{N}$ values (10.5 ± 1.1 ‰) to values previously recorded in subtropical waters for small pelagic and mesopelagic fishes such as myctophids (e.g. *Lampanyctodes hectoris* 10.6 ± 0.4 ‰, *Maurolicus muelleri* 10.2 ± 0.3 ‰, *Symbolophorus barnadi* 10.1 ± 0.8 ‰, *Diaphaus danae* 9.6 ± 0.7 ‰, Davenport & Bax 2002). Similar $\delta^{15}\text{N}$ values to that of the myctophid species may indicate that myctophids are less numerically important in the diet of *M. ingens* inhabiting subtropical waters. Guerreiro et al. (2015) also suggested that myctophids might not dominate the diet of *M. hyadesi* in the southern Indian Ocean as much as previously thought based on their similar $\delta^{15}\text{N}$ values. *M. ingens* also shared the lowest $\delta^{15}\text{N}$ value with *Teuthowenia pellucida* and *Ommastrephes bartrami*. The low $\delta^{15}\text{N}$ value of the large muscular ommastrephid *O. bartrami* in this study was somewhat surprising but may be due to a trophic plateau as has been documented by Parry (2008).

Taningia danae, in other cephalopod communities, has been reported as having one of the highest trophic values, similar to a sperm whale in the northeast Atlantic (Cherel et al. 2009a). However, in this study *T. danae* had relatively lower $\delta^{15}\text{N}$ values than some other cephalopod species but similar to the much smaller *Octopoteuthis* sp. The likely reason for the difference in trophic position is size-related as $\delta^{15}\text{N}$ values are positively correlated with DML (Cherel & Hobson 2005, Cherel et al. 2009b). *T. danae* beaks obtained from sperm whale stomachs from Tasmanian waters (Chapter 4) and the northeast Atlantic were considerably larger than in this present study. Beaks from Tasmanian sperm whales also support *T. danae* as being a high order predator (Chapter

4). The smaller sized *T. danae* in this study grouped with other species such as *T. filippovae* and *N. gouldi* that have a predominantly small pelagic or mesopelagic fish diet.

The ammoniacal squid, *I. cordiformis*, had the highest $\delta^{15}\text{N}$ values in this study and when corrected $\delta^{15}\text{N}$ values of this species (15.8 ‰) were compared with isotopic signatures of top predators from this region, it had higher $\delta^{15}\text{N}$ values than the sperm whales (14.6 ‰, Chapter 4) or pilot whales (12.2 ‰ Chapter 3). However, these corrected $\delta^{15}\text{N}$ values are lower than values recorded for the colossal squid *M. hamiltoni* in Tasmanian subtropical waters (16.8 ‰ Chapter 4), which is a top predator in these and Kerguelen waters (Cherel & Hobson, 2005). Mastigoteuthids are deep water pelagic or benthopelagic squid, equipped with tentacular clubs that have small suckers that act much like ‘fly paper’ to anything that touches them. The relatively high trophic level of *I. cordiformis* in this study appears to contradict the assumption that this family may feed on copepods or other small epibenthic zooplankton (Vecchione et al. 2014). Braid and Bolstad (2014) also obtained similar $\delta^{15}\text{N}$ values (15.5 - 16‰) for their specimens of *I. cordiformis*. When they analyzed the stomach contents using DNA barcoding they found the squid had fed on large pelagic birdbeak dogfish and snapper either actively or potentially by scavenging.

The prey of histioteuthids in subtropical waters is poorly understood, and indeed worldwide, despite their documented occurrence as the most important prey numerically in the diet of sperm whales stranded in this region and others (Evans & Hindell 2004a). The small variation in $\delta^{13}\text{C}$ values of *H. atlantica*, *H. macrohista* and *H. miranda* suggests that these three species live in closely related or overlapping habitats. Comparison of $\delta^{15}\text{N}$ values showed they were significantly different than one another, with *H. miranda* having the highest $\delta^{15}\text{N}$ values of all three species (11.7 ‰) and similar to *I. cordiformis* (12.0 ‰), the highest of any cephalopod in this study. *H. miranda* was approximately one trophic level higher than *H. macrohista* (8.3 ‰) with *H. atlantica* falling in between the two species (10.1 ‰) (Figure 2.6). It is interesting to note that the size of *H. atlantica* in this study based on LRL is the upper size of their bimodal distribution found in the diet of Antipodean wandering albatrosses *Diomedea antipodensis* and Gibson’s wandering albatrosses *D. antipodensis* from New Zealand Islands (Xavier et al. 2014). An examination of the C:N mass ratio reveals that *H.*

macrohista is also likely to be exhibiting a higher $\delta^{15}\text{N}$ value compared to the other two species due to a possible low chitin to protein ratio as discussed previously. Prior research has shown that histiotheuthids appear to predominantly feed on fish and crustaceans while supplementing with squid (Voss 1969). Myctophid fishes are recorded as being the predominant fish prey of *Histioteuthis celetaria*, which consumed similar amounts of crustaceans and fish (Voss et al. 1998). Likewise, Quetglas et al. (2010) documented that fish (predominantly myctophids), crustaceans and cephalopods, in that order, were the main prey of *Histioteuthis reversa* and *Histioteuthis bonnellii* from the Mediterranean Sea. Clarke (1980) found crustaceans, squid and unidentifiable remains in a few specimens of *H. miranda*. A diet of crustaceans and fish dominated by myctophids and supplemented by squid seems likely for at least *H. macrohista* and *H. atlantica* as they appear to have similar $\delta^{15}\text{N}$ values to other myctophid and crustacean eating squid such as *T. filippovae* and *N. gouldi* in the waters around Tasmania. It is probable that *H. miranda* may eat slightly larger fish or supplement their diet with larger fish or cephalopods to account for the higher $\delta^{15}\text{N}$ value similar to other top predators in the region.

There was no between species dependence on size with $\delta^{15}\text{N}$ values for this cephalopod community. These results suggest that trophic position is not size-structured. Conversely, results from other studies report some marine communities are trophically structured according to body size (Cohen et al. 1993; Mancini et al. 2014). In the present study, large specimens of *M. ingens* and *I. cordiformis* were at both the lower and higher end of the $\delta^{15}\text{N}$ continuum respectively. This corroborates that found for the cephalopod community in the northeast Atlantic (Cherel et al. 2009a). It also highlights the loose relationship between trophic position and body size and hence the complexity of web structuring within marine communities (Navarro et al. 2013).

In summary, this study supports the view proposed by Coll et al. (2013) that squids predominantly occupy central to high trophic positions in the marine food chain providing an important link from the micro-nekton to higher level consumers. They are generalist feeders that source food from all trophic levels. Due to their abundance and diverse habitats it is expected that any variable affecting cephalopod populations may impact both the top-down flow from cephalopods to prey and from the bottom-up since they are key prey resources for a variety of predators. Isotopic analysis is an important

tool for analyzing the functional relationships between predator and prey. Isotopes can be further used to document the association between important dietary parameters in cephalopods and the subsequent relationship to environmental variability.

CHAPTER THREE

DETERMINING THE ROLE OF CEPHALOPODS IN THE DIET OF PILOT WHALES IN TASMANIAN WATERS – WHAT DO STABLE ISOTOPES TELL US?

Abstract

Numerically, long-finned pilot whales (*Globicephala melas edwardii*) have one of the highest stranding rates in the world off the coast of Tasmania, Australia. Despite this, there is a paucity of documented information on their trophic dynamics. Stable isotopes of carbon and nitrogen were used to infer pilot whale foraging ecology based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the skin of whales from 3 mass strandings (Maria Island, King Island and Marion Bay) off the coast of Tasmania, Australia ($n = 147$). Intrinsic factors such as age, sex or lactation status showed little or no effect on skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. However, there was a small but significant gradient in $\delta^{13}\text{C}$ values between strandings with Maria Island ($-16.9 \pm 0.2 \text{ ‰}$) being the most enriched in ^{13}C and Marion Bay ($-17.8 \pm 0.2 \text{ ‰}$) being the least enriched. Likewise Maria Island whales had higher $\delta^{15}\text{N}$ values ($13.2 \pm 0.5 \text{ ‰}$) than King Island ($12.2 \pm 0.4 \text{ ‰}$) or Marion Bay ($12.0 \pm 0.4 \text{ ‰}$). The higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values found for Maria Island whales may suggest a demersal or shelf foraging habitat in comparison to the other two strandings that appear to reflect a more pelagic oceanic habitat. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cephalopod lower beaks ($n = 158$) comprising 9 different families collected from pilot whale stomachs were determined for 4 strandings around Tasmania (including Maria Island and Marion Bay). For the most part, cephalopod beaks had similar or more ^{13}C and ^{15}N enriched isotope values in comparison to the whales. This suggests the pilot whales may also be consuming other organisms lower in $\delta^{15}\text{N}$ values. Isotopic comparisons with other potential fish and cephalopod prey resources as well as other marine predators from the region highlighted the relatively low $\delta^{15}\text{N}$ value for pilot whales. The data does not support an exclusive diet of either cephalopods or fish. However, the pilot whales appear to show plasticity in their foraging strategy, although being largely teuthophagous, with a preference for oceanic squids.

INTRODUCTION

Assessing the diet and trophic relationship between top-level predators and their prey is essential for understanding the dynamics of the marine food chain. Furthermore, predator-prey relationships within a food web can be seminal in shaping the dynamics, productivity and stability of marine ecosystems (Young et al. 2015b). Marine mammals, including toothed whales, may play a key role in determining the structure and function of the marine food web due to their large size and subsequently high consumption rates of prey (Bowen 1997, Tollit et al. 2010). Therefore, pivotal to the conservation and management of toothed whale populations is the ability to identify their trophic linkages. This is primarily because the distribution of preferred prey species in both time and space, is likely to correlate with the movement, distribution and abundance of a predator within a specific habitat (MacLeod et al. 2003, Young et al. 2015a). Furthermore, field studies have indicated that reduced prey availability for some marine mammal predators has resulted in negative effects on their survival (Trites 2002).

Notwithstanding the importance of identifying and documenting trophic linkages between marine mammals and their prey, there is relatively limited information on some species, especially toothed whales, from some regions of the globe. This is likely due to the logistics of obtaining quantitative assessments of pelagic wide-ranging and deep-diving predators. Correspondingly, there is a paucity of dietary reconstruction data of toothed whales from the southern hemisphere, including from southern Australian waters around Tasmania, one of the world's stranding hotspots, with long-finned pilot whales *Globicephala melas edwardii* being one of the highest stranders numerically (Walters 2005, Bradshaw et al. 2006). Whaling in the 1900s in the Tasman Sea has provided an opportunity to obtain foraging behavior for some species such as the sperm whale *Physeter macrocephalus* (Clarke 1980, Clarke & MacLeod 1982). However, the largest proportion of current dietary information from this region is based on stomach content analysis of stranded animals (e.g. Gales & Pemberton 1992, Evans & Hindell 2004a). Analysis of stranded individuals provides a representation of recent feeding and may not accurately reflect the normal diet or indeed the long-term diet. Foraging assumptions may be based on feeding undertaken close to the stranding site rather than from their natural foraging habitats (Evans & Hindell 2004a, Pierce et

al. 2004). Furthermore, the stomachs of stranded whales are often empty or near empty thus precluding a complete dietary analysis (Pierce et al. 2004, Beatson et al. 2007a, b). Differential digestion as well as partial ingestion may lead to under- or over-representation of some prey (Pierce et al. 2004). Regardless of the limitations of stomach content analysis it remains an important technique that provides a key foundation for further ecological research using innovative biochemical dietary tracers that may help provide a greater resolution in trophic analysis (Tollit et al. 2010).

While dietary studies on marine mammals have greatly expanded in the past decade with the use of stable isotopes, there is still a critical need to better understand the foraging ecology of toothed whales, in particular long-finned pilot whales in the southern hemisphere. Recent isotopic studies have highlighted the trophodynamics of this species in other areas of the world including Northwest Iberia, Scotland, United Kingdom, United States and the Faroe Islands from Atlantic Waters (Monteiro et al. 2015a, b) as well as from Kerguelen waters in the Southern Indian Ocean (Fontaine et al. 2015). Additional dietary studies that have used stomach content analysis on stranded individuals were evaluated from around Portugal, Galicia and Scotland in northeast Atlantic waters (Santos et al. 2014). However, very little information exists for this species from subtropical waters around Tasmania. While all of these studies have suggested a predominantly cephalopod based diet or a catholic diet of cephalopods and fish for this species, a fatty acid analysis of this species from animals stranded around Tasmania suggested the possibility of a myctophid based diet (Walters 2005).

Current biochemical techniques used as dietary tracers in many marine mammals, including whales, are not only able to define predator-prey relationships in the trophic pathway but actually reconstruct the diet at varying resolutions. Since signatures of prey are reflected in the consumer, 'you are what you eat' underscores the foundation upon which techniques, such as fatty acid and stable isotope analysis are based (DeNiro & Epstein 1978, 1981, Young et al. 2015a). Stable isotope analysis of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) are common trophic analyses used extensively in many mammal, bird and fish studies (e.g. Ruiz-Cooley et al. 2004, Jaeger et al. 2013, Pethybridge et al. 2015). There is a predictable stepwise enrichment in ^{15}N between predator and prey with each

increasing trophic level. $\delta^{15}\text{N}$ values have been effectively used as a proxy for trophic position and indicator of an existing relationship between two organisms within the trophic pathway (e.g. Ruiz-Cooley et al. 2004, Herman et al. 2005). In contrast, although $\delta^{13}\text{C}$ differs geographically at the base of the food web, there is very little variation along the length of the food web. Consequently, the $\delta^{13}\text{C}$ value of an organism represents a signature of the primary source of the food in a region. Latitudinal gradients in $\delta^{13}\text{C}$ at the base of the food web along with inshore/offshore and pelagic/demersal $\delta^{13}\text{C}$ gradients provide essential clues in determining foraging and habitat useage by marine predators when the isotopic value of the consumer is compared to isotopically distinct geographical areas (Cherel & Hobson 2007). Stable isotope analysis of carbon and nitrogen on species from the stomach contents of a predator provide a greater understanding of the trophic ecology of not only the prey assemblage, but on the foraging ecology of the predator themselves (e.g. Cherel & Hobson 2005, Cherel et al. 2009a).

In recent years, stable isotope analysis using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ has been used to investigate the role of various intrinsic and extrinsic parameters influencing the trophic ecology of marine mammals. Habitat preference and trophic position as well as niche overlap were documented for a variety of mammals (e.g. Auriolles-Gamboa et al. 2013). Staudinger et al. (2014) highlighted with the use of stable isotopes the substantial niche overlap between pygmy and dwarf sperm whales. Conversely, divergent isotope ratios in North Pacific killer whales *Orcinus orca* were shown to represent dietary preferences consistent with three ecologically distinct populations (Herman et al. 2005). Additionally, temporal variation in diet was recorded for both narwhals *Monodon monoceros* (Watt & Ferguson 2015) and for female Australian fur seals *Arctocephalus pusillus doriferus* (Arnould et al. 2011). Individual differences related to sex, size and age have also been demonstrated to influence foraging strategies undertaken by male South American fur seals *Arctocephalus australis* (Vales et al. 2015) and beluga whales *Delphinapterus leucas* (Marcoux et al. 2012).

The aims of this study were to evaluate the trophodynamics of long-finned pilot whales *Globicephala melas edwardii* (hereafter referred to as pilot whales) from subtropical waters around Tasmania, Australia. Since direct feeding evaluations of these large odontocetes is logistically difficult, individuals from mass strandings, which are largely

free of bias due to disease, were used for this analysis. Four different objectives based on differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were used to examine (1) the effect of intrinsic factors such as age, size, sex and lactation status on dietary selection, (2) extrinsic factors such as temporal and spatial patterns between pilot whales from three separate strandings, (3) the trophic relationship of pilot whales in relation to other organisms in the pelagic food web of the Tasmanian ecosystem, and (4) isotope values of actual prey and potential prey of pilot whales to determine if they have a myctophid (as suggested by previous fatty acid analysis, Walters 2005) or a predominantly cephalopod based diet as has been documented in many regions of the world.

METHODS

Sample collection

Standardized stranding protocol (Geraci & Lounsbury 2005) performed by the Department of Primary Industries, Parks, Water and Environment (DPIPWE) for sample collections were undertaken on long-finned pilot whales that mass stranded on Maria Island and King Island (both November 2004) and on the beach at Marion Bay (October 2005) around Tasmania, Australia (Figure 3.1). Approximately 1cm² samples of skin were subsampled from large blocks of skin and blubber, which had immediately been packed on ice and later frozen and archived in a -20°C freezer at the DPIPWE laboratory in Hobart. Where possible, most skin subsamples were from larger samples taken dorsally on a standardized body site just anterior to the dorsal fin. However, with the exception of one animal, Maria Island samples were obtained from a lateral body site. Comparison samples of dorsal, lateral and ventral skin to calibrate between body regions were taken from four whales stranded at Maria Island. For each animal a straight-line total length (cm) from the tip of the rostrum to the deepest part of the notch in the tail fluke was performed. Sex as well as lactation status for females were determined by the presence or absence of milk after applying pressure to the mammary glands where a small incision into the whale just posterior to the teats had been made. Furthermore, each animal was assigned to an age/maturity class according to length and maturity (i.e. adult, subadult and juvenile) based on protocol established from Bloch et al. (1993). Age estimates on pilot whale teeth from the Marion bay stranding were performed at the DPIPWE (after Bloch et al. 1993). Stomach contents were collected only from the Marion Bay and Maria Island strandings, as well as two

additional strandings at Bicheno (September, 1992) and Ocean Beach (January 2006) (see Figure 3.1). Cephalopod beaks from stomach contents were kept in 70 % ethanol until further analysis. All cephalopod beaks were subsequently identified to species level where possible. Ommastrephid beaks were not identified to species level, as there are a number of species occurring in this region and they are a more difficult group to differentiate based on beak morphology. Lower rostral length was measured with digital calipers to the nearest mm.

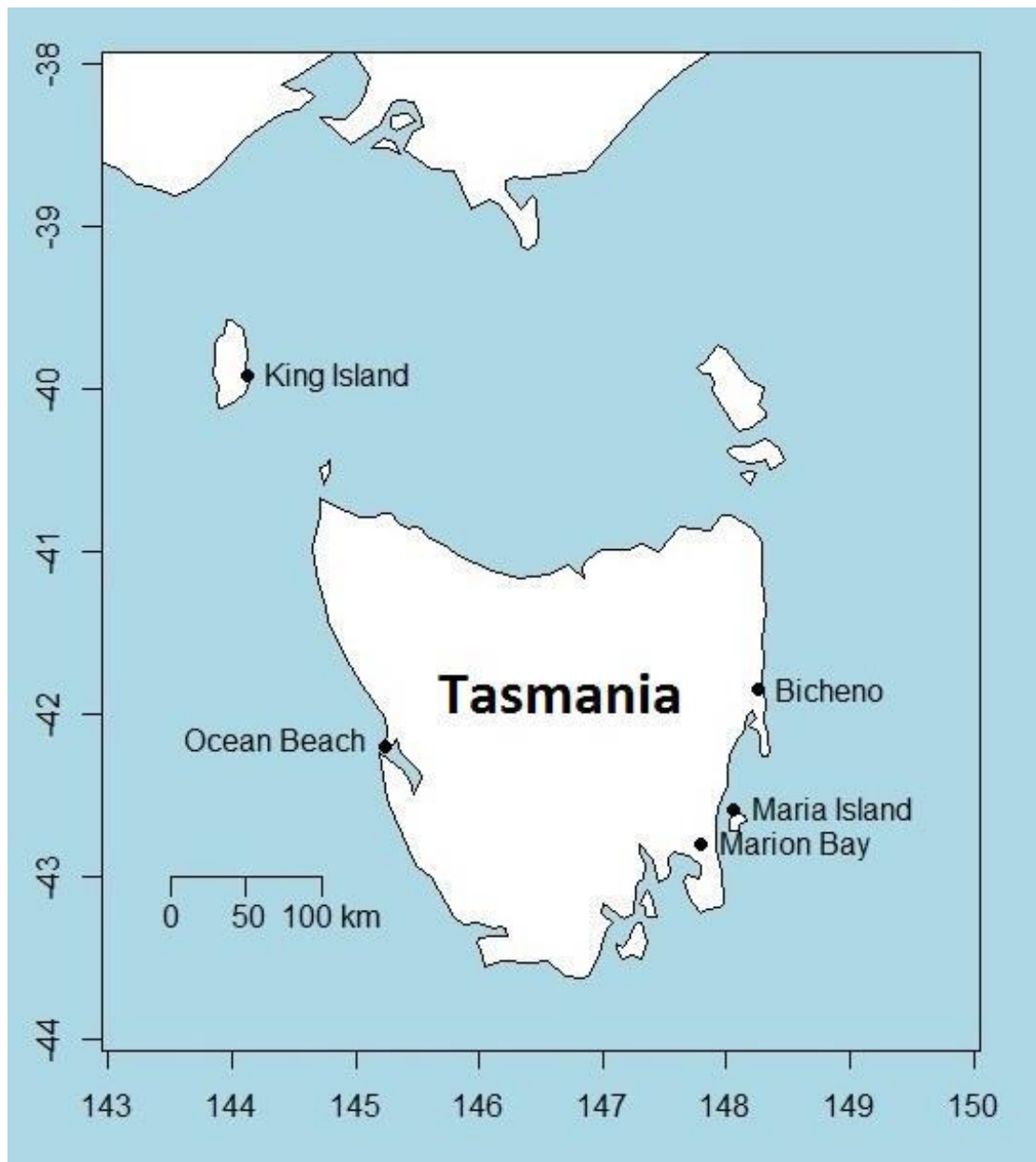


Figure 3.1. Map of Tasmania (southeastern Australia) showing stranded pilot whales (*Globicephala melas edwardii*) sampling sites.

The skin of 14 adult female sperm whales from a mass stranding at Croppies Beach off on North-eastern Tasmania (see Figure 4.1) in November, 2002 were also subsampled using the same protocol as that provided above for pilot whales, for predator comparisons within this ecosystem. Likewise, samples were also obtained for other potential predator competitors as well as potential prey resources. In January/February 2007 fish predator/resources were obtained from the local Hobart commercial fishing port that were collected off the southeast Tasmanian coast. However, redbait *Emmelichthys nitidus*, which were collected in February 2006, as well as jack mackerel *Trachurus declivis* and blue mackerel *Scomber australasicus* collected in June 2006 were obtained from waters off the east coast of Tasmania. Similarly, the myctophid *Lampanyctodes hectoris* was caught at a depth of 120 m on the shelf break off the east coast of Tasmania in October, 2006. All squid were collected incidentally each fishing season from the deepwater commercial trawl fishing industry operating off southern and eastern Tasmania from 2001 to 2006. Likewise, samples of *Sepioteuthis australis*, *Nototodarus gouldi*, *Sepia apama* and *Octopus maorum* were obtained from other commercial fishermen operating in nearshore Tasmanian waters (Chapter 2). Upon capture fish were packed on ice while cephalopods were either immediately frozen on board ship or packed fresh on ice until arrival at the port. Subsequently, the frozen squid were stored in a -20°C freezer until further analysis while the fresh squid and fish kept on ice were either frozen or immediately dissected.

Stable isotope analysis

Once defrosted, cephalopods and fish were identified to species level where possible. Fork length for fish and dorsal mantle length (DML) for squid were measured to the nearest mm. Prior to isotopic analysis lower and upper beaks were removed from whole squid and stored in 70% ethanol. Cephalopod beaks from stomach contents, which had been stored in 70% ethanol, from long-finned pilot whales were also identified to species level where possible (Clarke 1986, Xavier & Cherel 2009). All beaks were subsequently removed from ethanol and cleaned with distilled water. Lower rostral lengths (LRL) for all cephalopods were measured with digital calipers to the nearest millimeter (mm). A small section of the tip of the wing in the direction of growth was then sampled from each beak and rinsed with distilled water. The tip of the wing was selected as it signifies the most recent growth phase of the beak and therefore the

most recent somatic growth (Cherel & Hobson 2005). Beaks were not delipidated (after Cherel & Hobson 2005). For isotopic analysis beak wing tip samples were analyzed whole or cut in half for larger specimens. Where possible, approximately 10 replicate individuals were analyzed for each potential prey species as well as for the LRL size mode of beaks from pilot whale stomach contents.

Approximately 1 cm² pieces of anterior dorsal white muscle behind the head for fish and mid-anterior ventral mantle for squid (two species) were dissected and cleaned of any skin or mesentery and then rinsed with distilled water. A similar sized piece of pilot whale and sperm whale skin was also subsampled from larger frozen samples of skin and blubber. Any remaining subcutaneous adipose tissue was removed with a scalpel and the subsequent sample rinsed in distilled water. All samples were then frozen in preparation for isotopic analysis. Just prior to isotopic analysis samples were freeze dried, ground to a small powder with a Wig-L-Bug® and delipidated in cyclohexane. The ground whale skin, fish muscle or squid mantle samples along with 3 ml of cyclohexane were left to stand overnight in small covered test tubes under a fume hood. After delipidating overnight, samples were then centrifuged and the supernatant removed with a pipette. Samples were subsequently subjected to two more cyclohexane rinses. Between rinses the samples were allowed to stand for another 1 - 2 hours. Following delipidation samples were left to dry uncovered under a fume hood overnight. Relative abundance of carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) stable isotopes was determined by a Finnigan Delta Plus Advantage stable isotope-ratio mass spectrometer at the University of Victoria, British Columbia, Canada. For all isotopic analysis there was a 10 percent replication measurement for each isotopic sample run. The results of the isotopic analysis are presented in the usual δ notation relative to Vienna PDP belemnite for $\delta^{13}\text{C}$ and atmospheric N₂ (AIR) for $\delta^{15}\text{N}$. Replicate measurements of internal laboratory standards (DORM) indicated measurement errors of ± 0.1 ‰ and ± 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Statistical analysis

Repeated measures MANOVA was used to determine any differences between different body regions for skin collection (i.e. dorsal, lateral and ventral) on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values simultaneously. Moreover, ANOVA was used to confirm sexual dimorphism in pilot whales by examining if the body length of all animals was dependent on sex.

Correlational analysis on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with length as well as age was used to determine if these intrinsic factors were influential in the foraging strategy of long-finned pilot whales. Similarly, ANOVA was used to determine if there were any ontogenetic differences between assigned age/maturity classes (adults, subadults and juveniles). Furthermore, the effect of diet due to lactation, as expressed by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, was also examined in adult/subadult females using ANOVA. Two-way factorial ANOVAs with sex and stranding as the factors of interest were used to assess potential intrinsic and extrinsic (temporal and spatial) effects on average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values separately.

A comparison between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pilot whales for individual strandings and the squid beaks found in the stomachs of those animals were assessed using ANOVA to ascertain if the contents found in the stomachs reflected their diet. This was further assessed by examining the trophic discrimination factor, the difference between the consumer and its food (Bond & Hobson 2012), which is normally considered to be ~ 1 and in the range of 2 – 5 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively (Post 2002). The discrimination factor can also differ based on the species or the tissue examined. Different tissues have different turnover rates (or time for complete tissue renewal) that may range from days to weeks to months to years depending on the tissue examined (Tieszen et al. 1983, Caut et al. 2009, Borrell et al. 2013b). This would then reflect a different time period for dietary assimilation into tissues. Differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle and beak tissues from the same individuals of squid have been documented (Cherel & Hobson 2005, Chapter 2). Therefore, values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of cephalopod beaks were corrected to represent cephalopod muscle since beaks are approximately 0.7 ‰ more enriched in ^{13}C and 3.8 ‰ more depleted in ^{15}N (Chapter 2). Differences between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the same prey species from different strandings were also compared using t-tests to determine if whales from different strandings were foraging in similar areas. Pilot whale comparisons between potential squid and fish predators/resources from Tasmanian waters were analyzed with ANOVA on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values separately. Samples were assessed for violation of assumptions of the parametric tests, including normality. Given the uneven sample sizes, prey or predator species with less than three individuals were not used in any analysis. As much as possible similar sized animals were chosen for replication within a

species. Post-hoc Tukey's multiple comparisons were used when significant main or interaction effects were found.

RESULTS

Skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined for a total of 147 long-finned pilot whales from three strandings (King Island and Maria Island in November 2004 and Marion Bay, October 2005) around Tasmania. Skin sampling site (dorsal, lateral and ventral) showed no effect on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values when compared simultaneously (Wilks' Lambda $_{4,10} = 0.80$, $p > 0.05$). Skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranged from -18.4 to -16.6 ‰ and 11.3 to 14.3 ‰ across all strandings, respectively. Likewise, whales across all age/maturity classes ranged in body length from 190 to 608 cm (Table 3.1). Only animals from the Marion Bay stranding were aged and females ($n = 47$) ranged in age from 2 to 32 years, while males ($n = 25$) ranged in age from 2 to 23 years (Table 3.1).

Sexual dimorphism was evident in adults across all strandings ($F_{1,110} = 193.59$, $p < 0.0001$) with males being longer than females (550 ± 4 and 435 ± 3 cm, respectively). No association between body length and skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was evident when assessed for males and females separately across all strandings. Conversely, when Marion Bay pilot whales were analyzed for male and females separately, males revealed a moderate negative correlation between body length and $\delta^{13}\text{C}$ values ($r = -0.57$, $p = 0.004$, $n = 30$). Females only exhibited evidence of a weak negative correlation between body length and $\delta^{15}\text{N}$ values ($r = -0.35$, $p = 0.006$, $n = 59$). Body length and tooth increment number (estimated age) were highly correlated for Marion Bay males ($r = 0.90$, $p = 0.000$, $n = 25$) and females ($r = 0.89$, $p = 0.000$, $n = 59$). Increment number however, did not correlate with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in either sex. Across all strandings $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were correlated for both males ($r = 0.79$, $p = 0.000$, $n = 45$) and females ($r = 0.69$, $p = 0.000$, $n = 96$). Conversely, within individual strandings only the Marion Bay males resulted in a correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($r = 0.51$, $p = 0.004$, $n = 30$).

No significant differences between allocated age/maturity classes (adults, subadults and juveniles) were found for skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values across all strandings (both $p > 0.8$) and within the Marion Bay stranding (both $p > 0.2$) where there were sufficient numbers for comparisons (Table 3.1). Subsequently, all whales were pooled within

strandings for the factorial ANOVA on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with sex and stranding as the factors of interest. Pilot whales exhibited a significant sex x stranding interaction when comparing $\delta^{13}\text{C}$ ($F_{2,136} = 7.03$, $p = 0.001$) and $\delta^{15}\text{N}$ ($F_{2,136} = 6.04$, $p = 0.003$) values separately. Although the sex x stranding interaction accounted for 10% of the variation in $\delta^{13}\text{C}$ values, of that 10%, location and sex accounted for 76% and < 1%, respectively. Tukey's post-hoc multiple comparison tests using a new sex x location variable revealed that based on $\delta^{13}\text{C}$ values all strandings were significantly different from one another. There was a gradient in $\delta^{13}\text{C}$ values with Maria Island whales having the highest values ($-16.9 \pm 0.2 \text{ ‰}$) and Marion Bay ($-17.8 \pm 0.2 \text{ ‰}$) having the lowest, while King Island ($-17.3 \pm 0.2 \text{ ‰}$) had intermediate values. Any differences due to sex also depended on stranding with King Island and Maria Island males and females exhibiting similar $\delta^{13}\text{C}$ values. However, although multiple comparisons showed that Marion Bay females had significantly higher $\delta^{13}\text{C}$ values than males (0.2 ‰), it is not considered biologically significant. Similarly, as for $\delta^{13}\text{C}$, location accounted for the largest amount of variation (63%) in $\delta^{15}\text{N}$ values with the sex x location interaction and sex accounting for 8% and 9% of that variation in $\delta^{15}\text{N}$ values, respectively. Tukey's post-hoc multiple comparison tests using a sex x location variable showed evidence of differences in $\delta^{15}\text{N}$ values due to sex were also related to stranding. All strandings were significantly different from one another with the respective males and females having similar values, except for Maria Island males and females that also segregated from each other. Although King Island females segregated according to stranding, they also had similar values to Marion Bay male and female whales (see Table 3.1).

Adult and subadult female whales from the Marion Bay and King Island strandings showed no evidence of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values being dependent on lactation status (ANOVA, all $p > 0.4$). There were insufficient numbers of females from Maria Island with recorded lactation status to include in the analysis.

The range in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the King Island (0.9 and 1.7 ‰, respectively) and Maria Island (0.8 and 1.7 ‰, respectively) strandings were very similar. Conversely, while Marion Bay whales exhibited a similar range in foraging locations (0.8 ‰ for $\delta^{13}\text{C}$ values) they exhibited a greater range in $\delta^{15}\text{N}$ values (2.9 ‰).

Stomach contents

Cephalopod lower beaks ($n = 158$), comprising 9 different families, were collected from pilot whale stomachs from four different strandings around Tasmania (Bicheno, Ocean Beach, Maria Island and Marion Bay) from 1992 to 2005. Only whales from the Maria Island and Marion Bay strandings were also used for skin isotope analysis (see previous section). These beaks represented the predominant cephalopod species found in the stomachs of the whales in this study ($> 95\%$) across all strandings (Table 3.2).

Carbon isotopes

Beak $\delta^{13}\text{C}$ values of cephalopods from the stomach content of whales across all strandings ranged from -16.5‰ (*Architeuthis dux*, Bicheno and Marion Bay) to $-18.6 \pm 0.8\text{‰}$ (*Lycoteuthis lorigera*, male pilot whales Marion Bay), with an overall mean difference of 2.1‰ . For individual strandings mean overall differences in $\delta^{13}\text{C}$ values of the beaks were 1.4‰ for Bicheno, 1.7‰ for Ocean Beach and 2.1‰ for Marion Bay. Whales from the Maria Island stranding only contained ommastrephid beaks. When compared between strandings *Histioteuthis atlantica* had significantly higher $\delta^{13}\text{C}$ values from Bicheno ($-17.4 \pm 0.6\text{‰}$) than the Ocean Beach ($-18.3 \pm 0.3\text{‰}$) stranded whales ($U = 9$, $p = 0.015$). However, no significant differences were found between $\delta^{13}\text{C}$ values for *Ancistocheirus lesueuri* or *L. lorigera* beaks ($p > 0.05$). Ommastrephids, which were found in whale stomachs from all four strandings differed in their $\delta^{13}\text{C}$ values ($F_{6,43} = 9.9$, $p < 0.0001$). Smaller beaks from Ocean Beach whales had the lowest $\delta^{13}\text{C}$ values ($-17.8 \pm 0.3\text{‰}$) while larger beaks from Ocean Beach whales had the highest values ($-16.6 \pm 0.2\text{‰}$) with an overall difference of 1.2‰ . Between these two samples there was a graduating and overlapping continuum of $\delta^{13}\text{C}$ values (see Figure 3.2).

Table 3.1. Age class (based on length, mm), sex, total length (mm), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C:N mass ratio values of pilot whale (*Globicephala melas edwardii*) skin from three strandings around Tasmania, Australia (Marion Bay, King Island and Maria Island). All values are means \pm SD. ND = no data. Numbers in brackets indicate where numbers are less than the total n.

Stranding	Age/maturity class	Sex	n	Total length (cm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N (mass ratio)
Maria Island (November 2004)	Adults	Both	18	470 \pm 53	-16.9 \pm 0.2	13.4 \pm 0.4	3.7 \pm 0.1
		Males	6	533 \pm 45	-16.9 \pm 0.3	13.9 \pm 0.3	3.7 \pm 0.1
		Females	12	438 \pm 12	-16.9 \pm 0.1	13.2 \pm 0.3	3.8 \pm 0.1
		Lactating	2	448 \pm 8	-16.7 \pm 0.2	13.1 \pm 0.0	3.7 \pm 0.2
		Non-lactating	8	439 \pm 10	-16.8 \pm 0.1	13.3 \pm 0.4	3.8 \pm 0.1
	Subadults	Both	1	415	-16.8	13.0	3.8
		Males	1	415	-16.8	13.0	3.8
		Females	0				
	Juveniles	ND	0				
	All classes		19	467 \pm 53	-16.9 \pm 0.2	13.2 \pm 0.5	3.7 \pm 0.1
King Island (November 2004)	Adults	Both	31	455 \pm 52	-17.4 \pm 0.2	12.2 \pm 0.4	3.8 \pm 0.1
		Males	6	548 \pm 17	-17.3 \pm 0.2	12.7 \pm 0.4	3.7 \pm 0.1
		Females	21	437 \pm 24	-17.4 \pm 0.2	12.0 \pm 0.3	3.8 \pm 0.2
		Lactating	10	434 \pm 16	-17.4 \pm 0.2	12.0 \pm 0.3	3.8 \pm 0.2
		Non-lactating	11	439 \pm 30	-17.4 \pm 0.2	12.0 \pm 0.3	3.8 \pm 0.1
	Subadults	Both	4	323 \pm 21	-17.2 \pm 0.1	12.1 \pm 0.4	3.8 \pm 0.2
		Males	2	320 \pm 15	-17.2 \pm 0.04	11.9 \pm 0.6	3.6 \pm 0.1
		Females	2	325 \pm 34	-17.3 \pm 0.2	12.3 \pm 0.1	3.9 \pm 0.1
	Juveniles	ND	1	264	-17.5	12.4	3.9
	All classes		36	435 \pm 71	-17.3 \pm 0.2	12.2 \pm 0.4	3.8 \pm 0.1

Marion Bay (October 2005)	Adults	Both	68	461 ± 64 (67)	-17.9 ± 0.2	11.9 ± 0.4	3.7 ± 0.1
		Males	15	557 ± 40	-17.9 ± 0.2	11.8 ± 0.2	3.6 ± 0.1
		Females	52	433 ± 38	-17.8 ± 0.2	12.0 ± 0.4	3.7 ± 0.1
		Lactating	23	437 ± 39	-17.8 ± 0.1	11.9 ± 0.2	3.7 ± 0.1
		Non-lactating	28	429 ± 37	-17.8 ± 0.2	12.0 ± 0.5	3.6 ± 0.1
	Subadults	Both	14	389 ± 44	-17.9 ± 0.2	12.1 ± 0.3	3.6 ± 0.1
		Males	13	394 ± 42	-17.9 ± 0.2	12.1 ± 0.3	3.6 ± 0.1
		Females	1	328	-17.9	12.1	3.6
	Juveniles	ND	7	247 ± 33	-17.8 ± 0.3	12.2 ± 0.5	3.6 ± 0.1
	All classes		92	428 ± 88 (90)	-17.8 ± 0.2	12.0 ± 0.4	3.6 ± 0.1
All strandings			147	435 ± 81 (145)	-17.6 ± 0.4	12.2 ± 0.6	3.7 ± 0.1

Table 3.2. Beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, lower rostral length (LRL) and C:N mass ratio values of cephalopod species retrieved from the stomach contents of pilot whales (*Globicephala melas edwardii*) from four strandings around Tasmania, Australia (Bicheno, Marion Bay, Maria Island and Ocean Beach). All values are means \pm SD.

Family	Species	n	LRL (mm)	$\delta^{13}\text{C}$ (‰)	Adjusted $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Adjusted $\delta^{15}\text{N}$ (‰)	C:N (mass ratio)
Bicheno, September 1992								
Lycoteuthidae	<i>Lycoteuthis lorigera</i>	14	5.5 \pm 0.2	-17.9 \pm 0.6	-18.6 \pm 0.6	7.1 \pm 1.2	10.9 \pm 1.2	3.4 \pm 0.2
Enoploteuthidae	<i>Ancistrocheirus lesueuri</i>	13	8.3 \pm 0.2	-16.5 \pm 0.3	-17.2 \pm 0.3	10.4 \pm 0.4	14.2 \pm 0.5	3.3 \pm 0.2
Lepidoteuthidae	<i>Pholidoteuthis boschmai</i>	4	12.5 \pm 0.8	-17.4 \pm 0.3	-18.1 \pm 0.3	8.1 \pm 0.7	11.9 \pm 0.7	3.1 \pm 0.3
Architeuthidae	<i>Architeuthis dux</i>	1	8.3	-16.5	-17.1	10.7	14.5	3.2
Histioteuthidae	<i>Histioteuthis atlantica</i>	8	4.6 \pm 0.2	-17.4 \pm 0.6	-18.1 \pm 0.6	10.6 \pm 1.1	14.3 \pm 1.1	3.2 \pm 0.2
Ommastrephidae		10	10.5 \pm 0.3	-17.3 \pm 0.4	-18.0 \pm 0.4	8.4 \pm 1.4	12.2 \pm 1.4	3.2 \pm 0.3
Chiroteuthidae	<i>Chiroteuthis capensis</i>	6	5.4 \pm 0.2	-17.4 \pm 0.1	-18.1 \pm 0.1	10.0 \pm 0.6	13.8 \pm 0.5	3.1 \pm 0.2
Cranchiidae	<i>Teuthowenia pellucida</i>	13	4.4 \pm 0.2	-17.6 \pm 0.2	-18.2 \pm 0.2	9.0 \pm 0.9	12.8 \pm 0.9	3.3 \pm 0.3
	<i>Megalocranchia</i> sp.	2	7.5 \pm 0.4	-17.4 \pm 0.3	-18.1 \pm 0.3	9.7 \pm 0.3	13.5 \pm 0.3	3.5 \pm 0.1
Maria Island, November 2004								
Ommastrephidae	Ommastrephidae sp.	9	7.4 \pm 0.3	-16.9 \pm 0.3	-17.5 \pm 0.3	10.5 \pm 0.3	14.2 \pm 0.3	3.2 \pm 0.3
Marion Bay, October 2005								
Lycoteuthidae	<i>Lycoteuthis lorigera</i> (males)	9	5.3 \pm 0.3	-18.6 \pm 0.8	-19.3 \pm 0.8	6.1 \pm 1.6	9.9 \pm 1.6	3.4 \pm 0.2
	(females)	10	5.3 \pm 0.1	-18.0 \pm 0.8	-18.7 \pm 0.8	7.1 \pm 1.1	10.9 \pm 1.1	3.3 \pm 0.2
	<i>Lycoteuthis lorigera</i> (smaller)	19	5.3 \pm 0.2	-18.3 \pm 0.8	-19.0 \pm 0.8	6.6 \pm 1.5	10.4 \pm 1.4	3.4 \pm 0.2
		8	3.5 \pm 0.2	-18.5 \pm 0.4	-19.2 \pm 0.4	6.0 \pm 1.1	9.8 \pm 1.1	3.7 \pm 0.4
	<i>Ancistrocheirus lesueuri</i>	7	7.8 \pm 0.7	-16.7 \pm 0.7	-17.4 \pm 0.7	9.2 \pm 1.4	13.0 \pm 1.4	3.2 \pm 0.3
Enoploteuthidae	<i>Ancistrocheirus lesueuri</i>	7	7.8 \pm 0.7	-16.7 \pm 0.7	-17.4 \pm 0.7	9.2 \pm 1.4	13.0 \pm 1.4	3.2 \pm 0.3
Architeuthidae	<i>Architeuthis dux</i>	1	9.8	-16.5	-17.2	7.3	11.1	2.9
Ommastrephidae	Ommastrephidae sp. (large)	5	13.3 \pm 0.3	-16.9 \pm 0.3	-17.6 \pm 0.3	8.7 \pm 0.7	12.5 \pm 0.7	3.4 \pm 0.1
	(small)	7	7.2 \pm 0.6	-17.1 \pm 0.2	-17.8 \pm 0.2	11.1 \pm 0.8	14.8 \pm 0.7	3.2 \pm 0.2

Ocean Beach , January, 2006								
Octopoteuthidae	<i>Octopoteuthis</i> sp.	4	14.9 ± 0.7	-17.4 ± 0.3	-18.0 ± 0.3	11.5 ± 0.8	15.3 ± 0.8	3.3 ± 0.2
Histioteuthidae	<i>Histioteuthis atlantica</i>	8	4.3 ± 0.3	-18.3 ± 0.3	-18.9 ± 0.3	10.0 ± 0.8	13.7 ± 0.8	3.3 ± 0.2
Ommastrephidae	(small)	9	9.5 ± 0.3	-17.8 ± 0.3	-18.5 ± 0.3	7.8 ± 1.2	11.6 ± 1.2	3.4 ± 0.3
	(medium)	5	12.5 ± 0.2	-17.3 ± 0.6	-18.0 ± 0.6	8.3 ± 1.6	12.1 ± 1.7	3.2 ± 0.2
	(large)	5	14.2 ± 0.4	-16.6 ± 0.2	-17.3 ± 0.2	9.1 ± 0.8	12.9 ± 0.8	3.4 ± 0.2

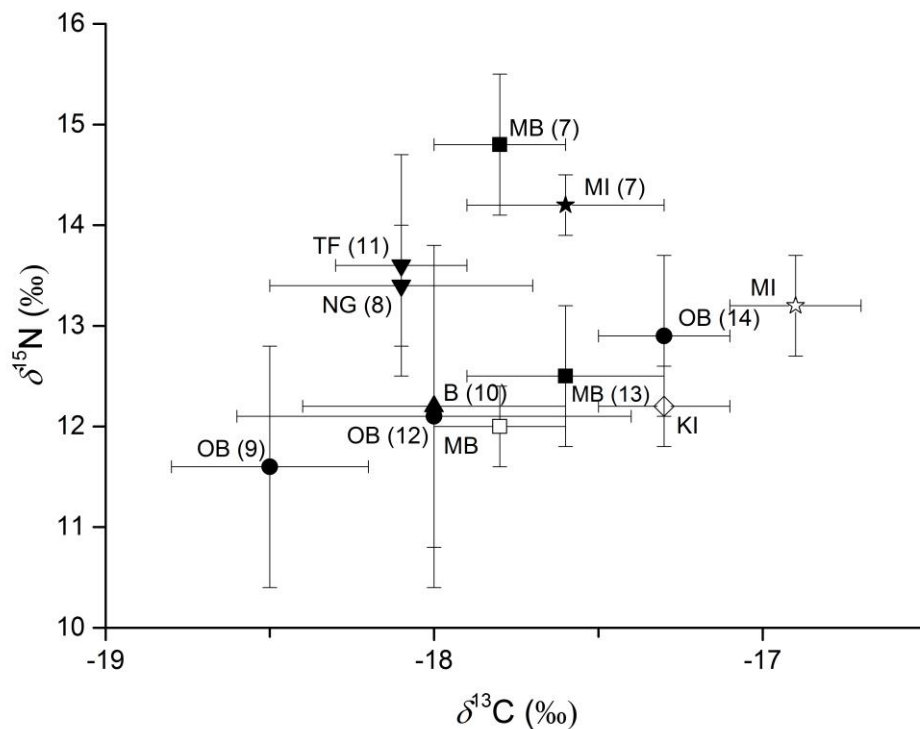


Figure 3.2. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for Maria Island (MI), King Island (KI) and Marion Bay (MB) pilot whale (*Globicephala melas edwardii*) skin with Ommastrephidae sp. beaks (corrected values, see Methods) from stomach contents of different size modes and from four different strandings (B, Bicheno; OB, Ocean Beach; MI, Maria Island; MB, Marion Bay). Values in parentheses represent mean lower rostral length (LRL) of beaks (mm). Beaks from two common ommastrephids (TF, *Todarodes filippovae*; NG *Nototodarus gouldi*) found in Tasmanian waters were included for comparison. The open symbols represent whale skin while the corresponding similar closed symbols represent Ommastrephidae sp. beaks from the respective whale stomach where applicable.

Corrected $\delta^{13}\text{C}$ values of all squid prey from whale predator stomachs from all four strandings (Bicheno, Marion Bay, Maria and Ocean Beach) differed from pilot whale skin values for $\delta^{13}\text{C}$ of the Maria Island ($F_{10,162} = 32.46$, $p < 0.0001$) King Island ($F_{10,179} = 30.93$, $p < 0.0001$) and Marion Bay ($F_{10,235} = 29.1$, $p < 0.0001$) strandings. Tukey's multiple comparison tests revealed that Maria Island had similar $\delta^{13}\text{C}$ values to *A. lesueuri* ($p > 0.05$) but all other squid were significantly more enriched in ^{13}C than their whale predators (all $p < 0.01$). King Island whale skin shared similar $\delta^{13}\text{C}$ values to *A. lesueuri*, large and small ommastrephids, *Octopoteuthis* sp. and *Pholidoteuthis boschmai*

(all $p > 0.05$) but had higher values than all other squids (*Chiroteuthis veranyi*, *L. lorigera*, medium ommastrephids, *Teuthowenia pellucida*, *H. atlantica* (all $p < 0.05$). Moreover, Marion Bay whales shared similar values to *C. veranyi*, large and small ommastrephids, *Octopoteuthis* sp., *P. boschmai* and *T. pellucida* (all $p > 0.05$). *A. lesueuri* was the only squid with higher $\delta^{13}\text{C}$ values ($p < 0.0001$) while *H. atlantica* and *L. lorigera* were significantly lower (both $p < 0.0001$) (Figure 3.3).

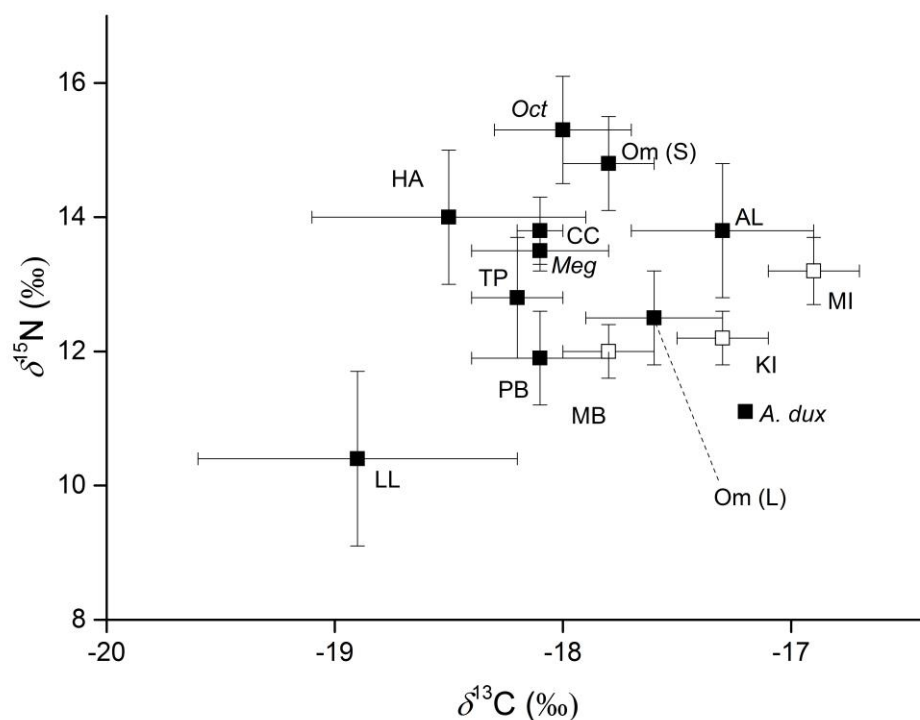


Figure 3.3. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ skin values for pilot whales (*Globicephala melas edwardii*) stranded at Maria Island (MI), King Island (KI) and Marion Bay (MB) (open symbols) in comparison to cephalopod beaks (corrected values, see Methods) from stomach contents (filled symbols) from all strandings (Bicheno, Maria Island, Marion Bay and Ocean Beach). AL, *Ancistocheirus lesueuri*; CC, *Chiroteuthis capensis*; HA, *Histioteuthis atlantica*; LL, *Lycoteuthis lorigera*; Meg, *Megalocranchia* sp.; Oct, *Octopoteuthis* sp.; Om (L), ommastrephids large; Om (S), ommastrephids small; PB, *Pholidoteuthis boschmai*; TP, *Teuthowenia pellucida*.

When comparing $\delta^{13}\text{C}$ values of skin from the Marion Bay stranding with only corrected $\delta^{13}\text{C}$ values from beaks from their respective stranding, values varied significantly

($F_{4,133} = 48.2$, $p < 0.0001$). Based on Tukey's post-hoc tests the pilot whales shared similar values to most squids (*A. lesueuri*, small and large ommastrephids) ($p > 0.05$) only segregating from the more depleted *L. lorigera* ($p < 0.0001$) (see Figure 3.4). Likewise, the $\delta^{13}\text{C}$ values of Maria Island whales ($-16.9 \pm 0.2 \text{ ‰}$) were significantly higher than the corrected $\delta^{13}\text{C}$ values of the ommastrephids ($-17.6 \pm 0.3 \text{ ‰}$) found in their stomachs ($t_{26} = 7.77$, $p < 0.0001$, 2 tailed) (see Figure 3.3).

Nitrogen isotopes

The mean $\delta^{15}\text{N}$ values of cephalopod beaks from pilot whale stomachs across all strandings ranged from $6.0 \pm 1.1 \text{ ‰}$ (*L. lorigera*, Marion Bay) to $11.5 \pm 0.8 \text{ ‰}$ (*Octopoteuthis* sp., Ocean Beach), with an overall difference in $\delta^{15}\text{N}$ values of 5.5 ‰ or equivalent to an estimated 1.5 - 2 trophic levels. The overall differences in $\delta^{15}\text{N}$ values for beaks for the Bicheno (3.5 ‰) and the Ocean Beach (3.7 ‰) stranding both approximated one trophic level. However, the greater overall difference in $\delta^{15}\text{N}$ values for the Marion Bay stranding (5.1 ‰) was only a little less than for all the cephalopod beaks from all strandings combined (5.5 ‰). There was no significant difference between $\delta^{15}\text{N}$ values for *H. atlantica*, *A. lesueuri* or *L. lorigera* when compared between strandings (all $p > 0.05$). In contrast, significant differences and a continuum of $\delta^{15}\text{N}$ values were indicated for ommastrephids within and between strandings ($H = 30.634$, $df = 6$, $p < 0.0001$). The overall mean difference between all ommastrephids was 3.2 ‰ (smaller Ocean Beach squid, LRL = $9.5 \pm 0.3 \text{ mm}$, $\delta^{15}\text{N} = 7.8 \pm 1.2 \text{ ‰}$ to smaller Marion Bay squid, LRL = $7.2 \pm 0.6 \text{ mm}$, $\delta^{15}\text{N} = 11.1 \pm 0.8 \text{ ‰}$). While there was a graduating increase in $\delta^{15}\text{N}$ values with increasing LRL for ommastrephids from the Ocean Beach stranding, those with the smallest LRLs from the Marion Bay and Maria Island strandings actually had the highest $\delta^{15}\text{N}$ values of all the ommastrephids, suggesting a mixture of species feeding at different trophic levels (Table 3.2, Figure 3.2).

Corrected $\delta^{15}\text{N}$ values of all squid prey from whale stomachs from all four strandings likewise segregated from the whale skin values for the Maria Island ($F_{10,162} = 33.89$, $p < 0.0001$), King Island ($F_{10,179} = 36.14$, $p < 0.0001$) and Marion Bay stranding ($F_{10,235} = 47.64$, $p < 0.0001$). Tukey's post-hoc multiple comparisons showed that while *Octopoteuthis* sp. had significantly higher $\delta^{15}\text{N}$ values than the Maria Island whales ($p < 0.05$), only *L. lorigera* and medium ommastrephids were significantly lower (both $p <$

0.01). Maria Island whales shared similar $\delta^{15}\text{N}$ values to *A. lesueuri*, *C. veranyi*, *H. atlantica*, *P. boschmai*, large and small ommastrephids and *T. pellucida* (all $p > 0.05$). Both King Island and Marion Bay whales shared similar $\delta^{15}\text{N}$ values to large and medium ommastrephids, *P. boschmai* and *T. pellucida* (all $p > 0.05$). Similarly, whales from both strandings were lower than *A. lesueuri*, *C. veranyi*, *H. atlantica*, small ommastrephids and *Octopoteuthis* sp. (all $p < 0.05$), but significantly higher than *L. lorigera* (both $p < 0.0001$) (Figure 3.3).

When the $\delta^{15}\text{N}$ values of the Marion Bay pilot whales were compared to the $\delta^{15}\text{N}$ values of beaks only retrieved from their stomachs they also varied ($F_{4,133} = 62.8$, $p < 0.0001$). Tukey's post-hoc multiple comparison tests revealed that the pilot whales shared similar values to the large ommastrephids ($p > 0.05$) but differed from all other beaks (all $p < 0.01$) with *L. lorigera* having significantly lower $\delta^{15}\text{N}$ values and the small ommastrephids and *A. lesueuri* having significant higher $\delta^{15}\text{N}$ values (Figure 3.4). Maria Island whales had a significantly lower $\delta^{15}\text{N}$ values (by -0.8‰) than the ommastrephid beaks from their respective stomachs ($t_{26} = -4.87$, $p < 0.0001$, 2-tailed) (Figure 3.3).

Potential cephalopod prey

Carbon isotopes

Skin of pilot whales from the three strandings (King Island, Maria Island and Marion Bay) and potential squid prey (using corrected $\delta^{13}\text{C}$) from Tasmanian waters differed in both their $\delta^{13}\text{C}$ ($F_{15,234} = 46.8$, $p < 0.0001$) and $\delta^{15}\text{N}$ values ($F_{15,234} = 83.9$, $p < 0.0001$). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranged from -19.2 ± 0.6 to $-16.9 \pm 0.2\text{‰}$ and from 10.5 ± 1.1 to $15.8 \pm 0.5\text{‰}$, respectively. Tukey's post-hoc multiple comparison tests revealed that skin $\delta^{13}\text{C}$ values from all strandings differed significantly from each other (all $p < 0.0001$) with the Maria Island stranding having the highest value and sharing a similar value to the neritic squid *S. australis* ($p > 0.05$). The King Island stranding did not segregate from inshore/demersal cephalopod species *Idioteuthis cordiformis*, *O. maorum* or *S. australis* ($p > 0.05$) but was significantly higher than all other squids ($p < 0.0001$). Moreover, the Marion Bay whales shared similar $\delta^{13}\text{C}$ values to *O. maorum*, *S. apama*, *N. gouldi*, *T. filippovae*, or *Taningia danae* (all $p > 0.05$) but *S. australis* and *I. cordiformis* were more ^{13}C enriched ($p < 0.05$). All other squids had significantly lower $\delta^{13}\text{C}$ values than Marion Bay whales (all $p < 0.05$) (Figure 3.5, Table 3.3).

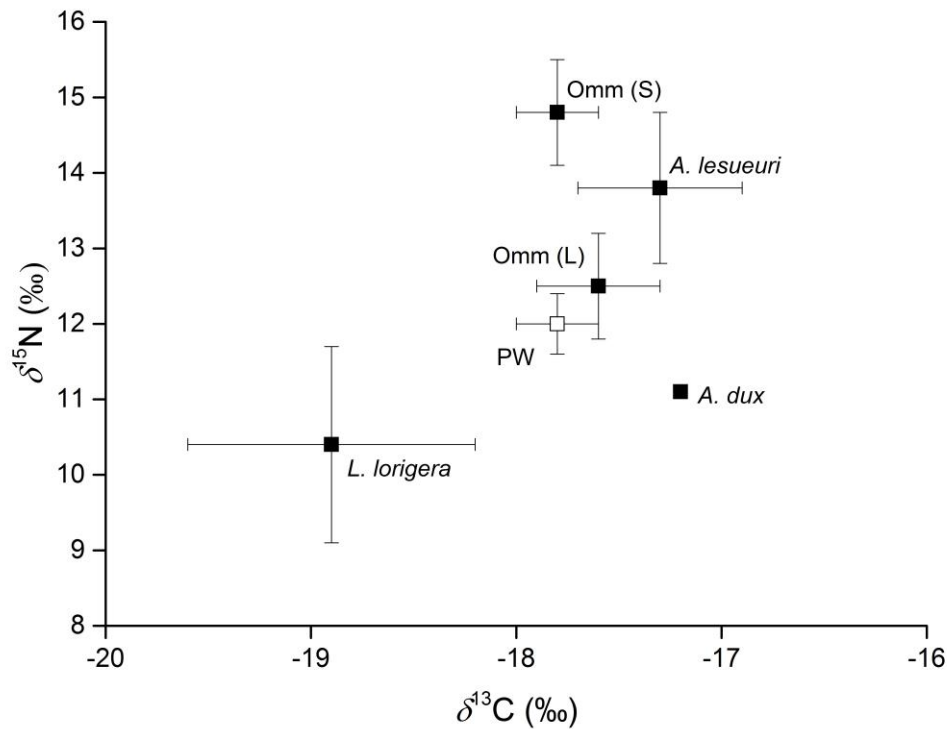


Figure 3.4 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ skin values for Marion Bay pilot whales (*Globicephala melas edwardii*) (PW, open symbol) and cephalopod beaks (corrected values, see Methods) from their stomach contents (closed symbols). Omm (L) and Omm (S) refer to large and small ommastrephids respectively.

Nitrogen isotopes

Skin of pilot whales from King Island, Maria Island and Marion Bay and potential squid prey (corrected $\delta^{15}\text{N}$ values) also differed in their $\delta^{15}\text{N}$ values ($F_{15,234} = 83.9$, $p < 0.0001$). Mean $\delta^{15}\text{N}$ values ranged from 10.5 ± 1.1 to 15.8 ± 0.5 ‰. Tukey's post-hoc multiple comparison tests revealed that only the $\delta^{15}\text{N}$ values of beaks from *M. ingens* and from the slightly smaller *T. pellucida* (Chapter 2) were significantly lower than pilot whale values from all strandings (all $p < 0.01$). Marion Bay and King Island pilot whales, which did not differ from each other, also shared similar $\delta^{15}\text{N}$ values to *Histioteuthis macrohista*, *S. apama*, *T. filippovae*, *T. danae* and *C. veranyi* (all $p > 0.05$). All other cephalopods (*N. gouldi*, *Histioteuthis atlantica*, *H. miranda*, *O. moarum*, *S. australis*, *I. cordiformis*) had significantly higher $\delta^{15}\text{N}$ values than the Marion Bay and King Island whales ($p < 0.0001$). Maria Island pilot whales shared comparable $\delta^{15}\text{N}$ values to *C. veranyi*, *H. atlantica*, *N. gouldi*, *O. maorum* and *T. danae* (all $p > 0.05$). However, only *T.*

filippovae, *S. apama*, *H. macrohista*, *T. pellucida* and *Moroteuthis ingens* were significantly more depleted in ^{15}N ($p < 0.0001$) than Maria Island whales while all others were more ^{15}N enriched (all $p < 0.0001$) (see Figure 3.5, Table 3.3).

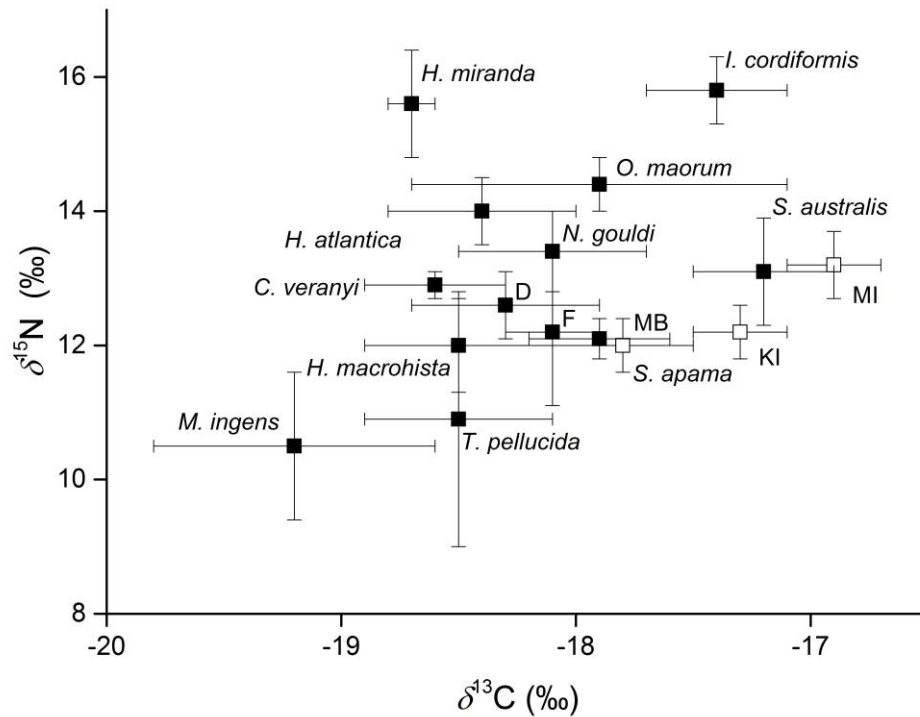


Figure 3.5. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ skin values for Maria Island (MI), King Island (KI) and Marion Bay (MB) pilot whales (*Globicephala melas edwardii*) (open symbols) with potential cephalopod resources from Tasmanian waters (filled symbols). Corrected values were used for cephalopod beaks (see Methods). D, *Taningia danae*; F, *Todarodes filippovae*

Potential fish prey

Carbon isotopes

Skin from pilot whales from the Maria Island, King Island and Marion Bay strandings and potential fish resources from Tasmanian waters differed in $\delta^{13}\text{C}$ values ($F_{19,286} = 108.86$, $p < 0.0001$). Mean $\delta^{13}\text{C}$ values ranged from -19.7 ± 0.3 to -16.4 ± 0.2 ‰. Post-

hoc multiple comparison tests highlighted that Maria Island whales had one of the highest $\delta^{13}\text{C}$ values with only gummy sharks *Mustelus antarcticus* being significantly higher ($p < 0.01$). The whales also shared a similar value to spikey oreos *Neocyttus rhomboidalis* ($p > 0.05$) but segregated from all other fish and whales (all $p < 0.01$). King Island whales shared similar values to blue grenadier *Macruronus novaezelandiae*, mirror dory *Zenopsis ebulosus*, *Nemadactylus macropterus*, pink ling *Genypterus blacodes* and ribaldo cod *Mora moro* ($p > 0.05$) with only gummy shark, spikey oreos and the Maria Island whales being significantly more enriched in ^{13}C (all $p < 0.01$) than the whales. All other fish had significantly lower $\delta^{13}\text{C}$ values than skin from the King Island whales (all $p < 0.01$). Skin samples from Marion Bay whales, which were significantly lower than both the King Island and Maria Island whales (both $p < 0.0001$), shared similar $\delta^{13}\text{C}$ values to blue grenadier, gemfish *Rexea solandri*, mirror dory, morwong, pink ling and ribaldo cod (all $p > 0.05$), but they segregated from the spikey oreos and gummy shark which were significantly more enriched in ^{13}C ($p < 0.0001$). All other fish segregated due to having significantly lower $\delta^{13}\text{C}$ values (all $p < 0.0001$) (see Figure 3.6, Table 3.3).

Nitrogen isotopes

Skin samples from pilot whales from the Maria Island, King Island and Marion Bay strandings and potential fish resources also differed in $\delta^{15}\text{N}$ values ($F_{19,286} = 92.0$, $p < 0.0001$). Mean $\delta^{15}\text{N}$ values ranged from 11.1 ± 0.4 to 15.4 ± 0.3 ‰. With the exception of redbait and *L. hectoris* (myctophid) which were significantly lower in ^{15}N than all the pilot whales and other fish (all $p < 0.01$), post-hoc tests showed that both King Island and Marion Bay pilot whales shared some of the lowest $\delta^{15}\text{N}$ values along with blue-eye trevalla *Hyperoglyphe antarctica* and white warehou (all $p > 0.05$). King Island whales also did not segregate from blue mackerel ($p > 0.5$). All other fish had significantly higher values than the King Island and Marion Bay whales (all $p < 0.0001$). Maria Island whales that were significantly more enriched in ^{15}N than whales from the other two strandings did not segregate from blue mackerel, jack mackerel, ocean perch *Helicolenus* sp., blue warehou *Seriolella brama*, silver warehouse *Seriolella punctata*, white warehou *Seriolella caerulea* and spikey oreo (all $p > 0.05$). Only redbait, *L. hectoris* and blue-eye trevalla had significantly lower $\delta^{15}\text{N}$ values (all $p < 0.0001$) than Maria Island whales. In contrast gummy shark, gemfish, morwong, mirror dory, ribaldo

cod, pink ling and blue grenadier had higher $\delta^{15}\text{N}$ values than Maria Island whales (all $p < 0.01$) (see Figure 3.6, Table 3.3).

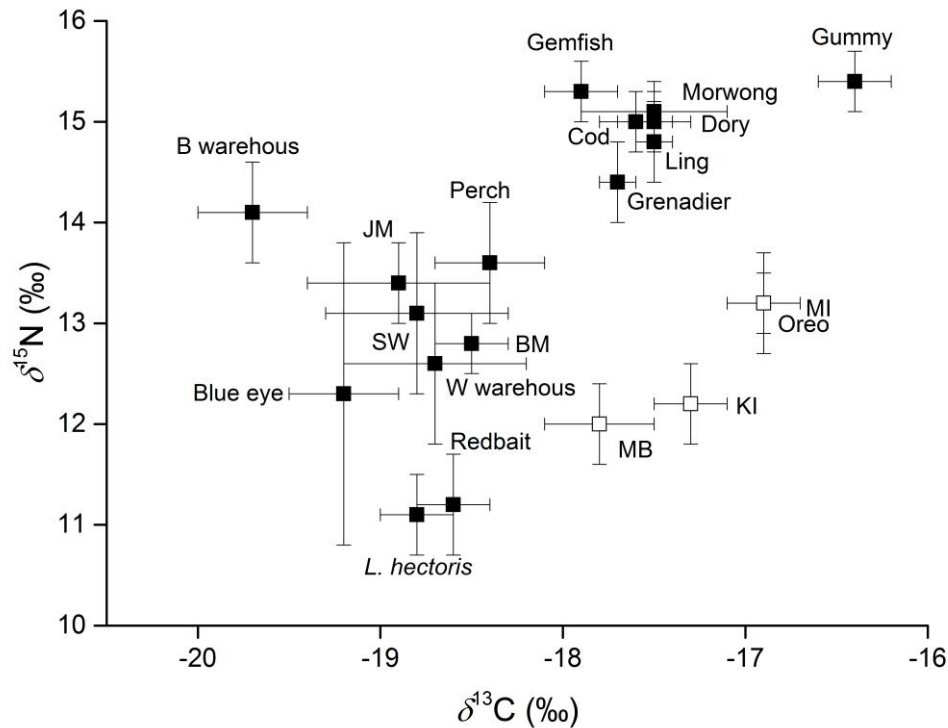


Figure 3.6. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ skin values for Maria Island (MI), King Island (KI) and Marion Bay (MB) pilot whales (*Globicephala melas edwardii*) (open symbols) with potential fish resources (white muscle, filled symbols). Blue eye, Blue eye trevalla *Hyperoglyphe antarctica*; grenadier, Blue grenadier *Macruronus novaezelandiae*; BM, Blue mackerel *Scomber australasicus*; B warehou, Blue warehou *Seriolella brama*; Dory, Mirror dory *Zenopsis ebulosus*; Gemfish *Rexea solandri*; morwong, Jackass morwong *Nemadactylus macropterus*; JM, Jack mackerel *Trachus declivis*; Perch, Ocean Perch *Helicolenus* sp.; Ling, Pink ling *Genypterus blacodes*; Redbait *Emmelichthys nitidus*; Cod, Ribaldo cod *Mora moro*; SW, Silver warehou *Seriolella punctata*; Oreo, Spikey oreo *Neocyttus rhomboidalis*; W warehou, White warehou *Seriolella caerulea*

Marine predator comparisons

Visual examination of pilot whale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to values from other marine mammals (extracted from Davenport & Bax 2002) highlighted that although the pilot whales had similar $\delta^{15}\text{N}$ values to some of the predators they had the lowest $\delta^{15}\text{N}$ values of all the predators. Sperm whales, which are known to be squid feeding specialists have an almost identical $\delta^{13}\text{C}$ value to the pilot whales, but are approximately one trophic level higher. Furthermore, two top predators in this region, killer whales *Orcinus orca* (although slightly more depleted in ^{13}C than the pilot whales) and the Australian fur seal (which has a similar $\delta^{13}\text{C}$ value to the Maria Island whales) are estimated to be 1 – 2 trophic levels above pilot whales. (Figure 3.7, see Table 3.3).

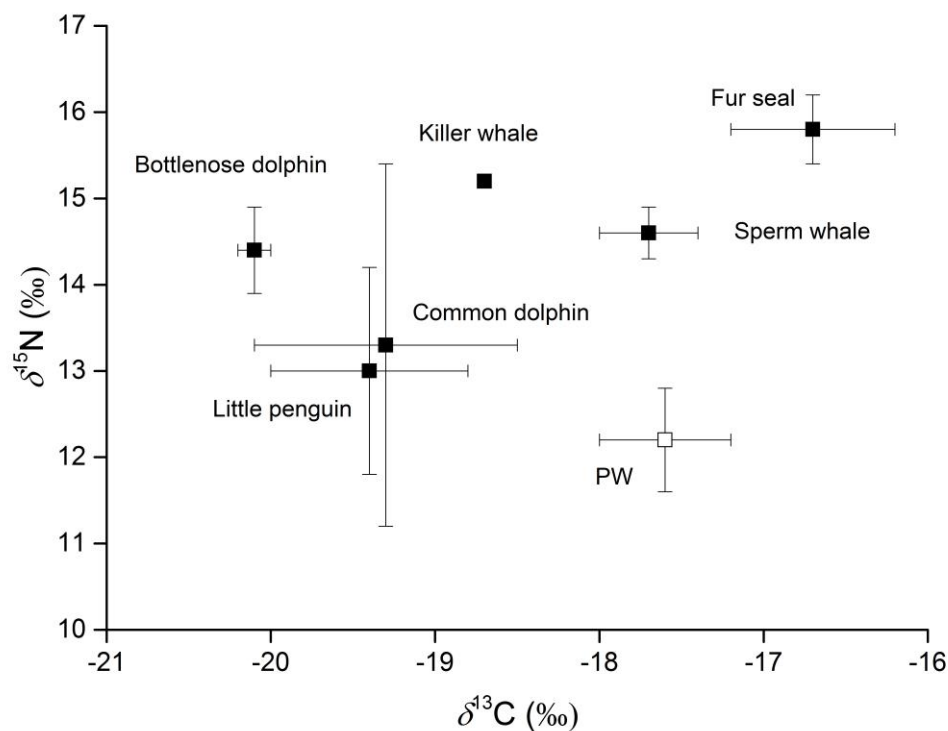


Figure 3.7. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ skin values of pilot whales (PW) (*Globicephala melas edwardii*) across all strandings (open symbol) in comparison with sperm whale skin and muscle from other predators (closed symbol). Marine mammals and little penguin values are taken from Davenport & Bax (2002).

Table 3.3. Skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pilot whales (*Globicephala melas edwardii*) in comparison to values from various organisms collected in Tasmanian waters, as well as from the Southeast Australian continental shelf (Davenport & Bax, 2002). All values are means \pm SD. Habitat code: P (pelagic), D (demersal), O (offshore), I (inshore).

Sample or species	Habitat	Tissue	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	N	Source
Pilot whale <i>Globicephalus melas edwardii</i>	P/O	skin	-17.6 ± 0.4	12.2 ± 0.6	147	This study
POM (particulate organic matter)			-21.5 ± 0.2	5.7 ± 1.4	91($\delta^{13}\text{C}$), 81($\delta^{15}\text{N}$)	Davenport & Bax, 2002
Zooplankton						
mixed			-21.3 ± 0.5	7.7 ± 1.9	6	Davenport & Bax, 2002
crustacean			-20.2	7.4	1	Davenport & Bax, 2002
Cephalopods						
<i>Sepia apama</i>	D/I	beak	-17.9 ± 0.3	12.1 ± 0.3	9	Chapter 2
<i>Idioteuthis cordiformis</i>	D/O	beak	-17.4 ± 0.3	15.8 ± 0.5	10	Chapter 2
<i>Sepioteuthis australis</i>	D/I	muscle	-17.2 ± 0.3	15.5 ± 0.2	9	Chapter 2
<i>Lycoteuthis lorigera</i>	P/O	beak	-18.9 ± 0.7	10.5 ± 1.3	41	This study
<i>Ancistocheirus lesueuri</i>	P/O	beak	-17.3 ± 0.4	13.8 ± 1.0	20	This study
<i>Octopoteuthis</i> sp.	P/O	beak	-18.0 ± 0.3	15.3 ± 0.8	4	This study
<i>Taningia danae</i> (medium)	P/O	beak	-18.3 ± 0.4	12.6 ± 0.5	3	Chapter 2
<i>Taningia danae</i> (large)	P/O	beak	-18.2	12.3	1	Chapter 2
<i>Moroteuthis ingens</i>	D/O	beak	-19.2 ± 0.6	10.5 ± 1.1	9	Chapter 2
<i>Pholidoteuthis boschmai</i>	P/O	beak	-18.1 ± 0.3	11.9 ± 0.7	4	This study
<i>Architeuthis dux</i>	P/O	beak	-17.2 ± 0.1	12.8 ± 2.4	2	This study
<i>Histioteuthis atlantica</i>	P/O	beak	-18.5 ± 0.6	14.0 ± 1.0	16	This study
<i>Histioteuthis macrohista</i>	P/O	beak	-17.8 ± 0.4	12.2 ± 0.9	9	Chapter 2
<i>Histioteuthis miranda</i>	P/O	beak	-18.7 ± 0.1	15.6 ± 0.8	11	Chapter 2
<i>Todarodes filippovae</i>	P/O	beak	-18.1 ± 0.2	12.2 ± 1.1	10	Chapter 2
<i>Nototodaros gouldi</i>	P/O/I	beak	-18.1 ± 0.4	13.4 ± 0.6	9	Chapter 2
<i>Chiroteuthis veranyi</i>	P/O	beak	-18.6 ± 0.3	12.9 ± 0.2	3	Chapter 2
<i>Ommastrephes bartrami</i>	P/O	beak	-16.3	10.9	1	Chapter 2
<i>Chiroteuthis capensis</i>	P/O	beak	-18.1 ± 0.1	13.8 ± 0.5	6	This study
<i>Teuthowenia pellucida</i>	P/O	beak	-18.3 ± 0.2	12.8 ± 0.9	13	This study
smaller	P/O	beak	-17.9 ± 0.4	10.9	6	Chapter 2

<i>Megalocranchia</i> sp.	P/O	beak	-18.1 ± 0.3	13.5 ± 0.3	2	This study
<i>Octopus maorum</i>	D/O	beak	-17.8 ± 0.8	14.4 ± 0.4	5	Chapter 2
Elasmobranch						
Gummy shark <i>Mustelus antarcticus</i>	D/O	White muscle	-16.4 ± 0.2	15.4 ± 0.3	10	This study
Myctophids						
<i>Lampanyctoides hectoris</i> Hector's lanternfish	P/O	White muscle	-18.8 ± 0.2	11.1 ± 0.4	10	This study
<i>Symbolophorus barnardi</i>	P/O	White muscle	-19.4 ± 0.7	10.1 ± 0.8	5	Davenport & Bax, 2002
<i>Diaphus danae</i>	P/O	White muscle	-19.7 ± 0.8	9.6 ± 0.7	7	Davenport & Bax, 2002
<i>Gymnoscopelus piabilis</i>	P/O	White muscle	-19.3	11.7	1	Davenport & Bax, 2002
Other fish						
Ribaldo cod <i>Mora moro</i>	D/O	White muscle	-17.6 ± 0.2	15.0 ± 0.3	10	This study
Blue grenadier <i>Macruronus novaezelandiae</i>	P/O	White muscle	-17.7 ± 0.1	14.4 ± 0.4	9	This study
Pink ling <i>Genypterus blacodes</i>	D/O	White muscle	-17.5 ± 0.1	14.8 ± 0.4	10	This study
Mirror dory <i>Zenopsis ebulosus</i>	D/O	White muscle	-17.5 ± 0.2	15.0 ± 0.3	5	This study
Spikey oreo <i>Neocyttus rhomboidalis</i>	D/O	White muscle	-16.9 ± 0.2	13.2 ± 0.3	10	This study
Ocean Perch <i>Helicolenus</i> sp.	P/O	White muscle	-8.4 ± 0.3	13.6 ± 0.6	10	This study
Jack mackerel <i>Trachus declivis</i>	P/O	White muscle	-18.9 ± 0.5	13.4 ± 0.4	10	This study
Redbait <i>Emmelichthys nitidus</i>	P/O	White muscle	-18.6 ± 0.2	11.2 ± 0.5	10	This study
Jackass morwong <i>Nemadactylus macropterus</i>	D/O	White muscle	-17.5 ± 0.4	15.1 ± 0.3	10	This study
Gemfish <i>Rexea solandri</i>	P/O	White muscle	-17.9 ± 0.2	15.3 ± 0.3	6	This study
Blue mackerel <i>Scomber australasicus</i>	P/O/I	White muscle	-18.5 ± 0.2	12.8 ± 0.3	10	This study
Blue-eye trevalla <i>Hyperoglyphe antarctica</i>	D/O	White muscle	-19.2 ± 0.3	12.3 ± 1.5	11	This study
Blue warehou <i>Seriolella brama</i>	D/O	White muscle	-19.7 ± 0.3	14.1 ± 0.5	10	This study
White warehou <i>Seriolella caerulea</i>	D/O	White muscle	-18.7 ± 0.5	12.6 ± 0.8	7	This study
Silver warehou <i>Seriolella punctata</i>	D/O	White muscle	-18.8 ± 0.5	13.1 ± 0.8	11	This study
Sea bird						
Little penguin <i>Eudyptula minor</i>			-19.4 ± 0.6	13.0 ± 1.2	19	Davenport & Bax, 2002

Marine mammals

Australian fur seal <i>Arctocephalus pusillus doriferus</i>		muscle	-16.7 ± 0.5	15.8 ± 0.4	2	Davenport & Bax, 2002
Common dolphin <i>Delphinus delphis</i>	P/O	muscle	-19.3 ± 0.8	13.3 ± 2.1	3	Davenport & Bax, 2002
Killer whale <i>Orcinus orca</i>	P/O	muscle	-18.7	15.2	1	Davenport & Bax, 2002
Sperm whale <i>Physeter macrocephalus</i>	P/O	skin	-16.7 ± 0.3	15.4 ± 0.3	14	This study
Bottlenose dolphin <i>Tursiops truncatus</i>	P/O/I	muscle	-20.1 ± 0.1	14.4 ± 0.5	5	Davenport & Bax, 2002

DISCUSSION

Intrinsic variation

Pilot whales in this study revealed very little variation in skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values within and between strandings due to intrinsic factors such as age, size or sex despite males and females being size-dimorphic. These factors normally would be expected to affect dietary selection based on the greater physiological demands on the larger males due to size or on females due to reproductive status. However, dietary shifts after weaning have been documented for juveniles of other marine mammals (e.g. South American fur seals, Vales et al. 2015, and grey seals *Halichoerus grypus*, Tucker et al. 2007). Likewise, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ retrospective analysis on teeth from killer whales (Newsome et al. 2009) and sperm whales (Mendes et al. 2007b) has recognized ontogenetic shifts in diet from juveniles to adult. In this study a moderate negative relationship between $\delta^{13}\text{C}$ values and size was found for male pilot whales from the Marion Bay stranding. This may suggest that larger sized males may be diving deeper in the water column as proposed for false killer whales *Pseudorca crassidens* (Riccialdelli & Goodall 2015). However, similar to the results found by Fontaine et al. (2015) for pilot whales in Kerguelen waters, pilot whales from Tasmanian waters showed no major variations in foraging as they matured.

The $\delta^{15}\text{N}$ values of juvenile pilot whales in this study did not follow the expected theory of an ontogenetic isotopic shift to the lower nitrogen values of subadult or adult values. Within and across all strandings, juveniles had the same $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as all other age/maturity classes. Although a mother's milk has a similar $\delta^{13}\text{C}$ value to the calf (Newsome et al. 2009), a whole trophic level enrichment theory based on $\delta^{15}\text{N}$ values has been proposed where juveniles may be a trophic level above their mothers due to the juveniles 'consuming' their mothers (Jenkins et al. 2001). However, for a number of mammalian species the $\delta^{15}\text{N}$ values during lactation have not supported a general trophic enrichment based on $\delta^{15}\text{N}$ values in plasma between mothers and their nursing offspring, but rather may be species (Jenkins et al. 2001) or tissue specific (Habran et al. 2010) or even related to nutritional stress (Valenzuela et al. 2010). However, it has been suggested that when the milk of a mother is compared to the juvenile's tissue, the trophic enrichment based on $\delta^{15}\text{N}$ values is more visible (Cherel et al. 2015). It is

possible that the juveniles from the Tasmanian study were closer to being weaned and were consuming some prey rather than being wholly dependent on milk. As during the weaning process, the $\delta^{15}\text{N}$ values of the juvenile should theoretically approach that of its mother relative to the proportion of its food mimicking the adult diet (Jenkins et al. 2001). However, Browning et al. (2014) found that independently feeding juvenile dolphins had significantly higher $\delta^{15}\text{N}$ values than adults even when fed the same diet. Nevertheless, Monteiro et al. (2015b) found that unweaned individual pilot whales with a body length less than 219 cm were enriched in ^{15}N compared to larger pilot whales. Similarly, Fontaine et al. (2015) confirmed in their study that smaller long-finned pilot whales from Kerguelen waters generally had higher $\delta^{15}\text{N}$ values for skin and muscle compared to larger whales.

Some sexually dimorphic cetaceans display sex-specific foraging strategies due to differing physiological needs or habitat use. This is particularly evident in sperm whales where differences in the diet of males and females based on stomach content analysis and retrospective isotopic analysis of teeth have been documented (Evans & Hindell 2004a, Mendes et al. 2007a, b). This may possibly be due to sex-specific foraging strategies based on segregation of males from the natal pod after a certain age (Flinn et al. 2002, Mendes et al. 2007a). In contrast, little evidence for sex-related isotopic differences were evident for false killer whales based on retrospective analysis of teeth, suggesting there was no resource partitioning between males and females (Riccialdelli & Goodall 2015). While a portion of the differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be attributed to sex differences in this study, the percent variation attributed to this factor is relatively small, particularly for $\delta^{13}\text{C}$. Fontaine et al. (2015) likewise found that sex did not contribute to the variation in $\delta^{13}\text{C}$ values and only a small amount to the variation in $\delta^{15}\text{N}$ values of pilot whales from Kerguelen waters. The variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were also found to be independent of sex for pilot whale populations from the Strait of Gibraltar (de Stephanis et al. 2008) and Atlantic waters (Monteiro et al. 2015b).

In this study, lactation also failed to influence the trophic status of adult females when comparing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lactating to non-lactating individuals. Due to the high energy requirements of lactation in mammals, there is either a concomitant increase in food consumption or a shift in dietary habits (Valenzuela et al. 2010). Based

on lipid analysis of pilot whales from Tasmanian waters, Walters (2005) documented that blubber thickness, lipid content and lipid composition was likewise not dependent on reproductive status. It was suggested that pilot whales employ a reproductive strategy of continuous resource acquisition throughout the high energetic demands of reproduction and lactation, similar to other highly social whales such as sperm whales (Evans et al. 2003a).

Extrinsic variation

While individual differences in trophic ecology of marine mammals often account for some differences in the variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, much of the variation in this study can be attributed to spatial or temporal differences. Differences in $\delta^{13}\text{C}$ values help to delineate foraging strategies and habitats. Geographical variation in the foraging habitats of marine predators can be attributed to variation in $\delta^{13}\text{C}$ values at the base of the food web due to latitudinal, inshore/offshore or pelagic/benthic gradients (e.g. Cherel & Hobson 2007). Inshore or demersal habitats are more ^{13}C enriched than offshore or pelagic habitats due to higher nutrient concentrations and subsequently greater productivity (France 1995, McMahon et al. 2013).

A small but significant overall gradient in $\delta^{13}\text{C}$ values (+ 0.9 ‰) was found across the three strandings in this study. Maria Island whales were the most enriched in ^{13}C . These whales shared values similar to the oceanic demersal fish, spikey oreo, or neritic squid, *S. australis*. If whale skin is corrected to reflect muscle tissue, since whale skin is slightly more enriched in ^{13}C than muscle (+ 0.7 ‰, Fontaine et al. 2015) it is likely that the $\delta^{13}\text{C}$ values will reflect more closely other neritic (*O. maorum* and *S. apama*) or demersal (*I. cordiformis*) cephalopod species as well. Unadjusted $\delta^{13}\text{C}$ skin values are also similar to values documented for the neritic Australian fur seal (Davenport & Bax, 2002) that feeds predominantly over the shallow shelf of Bass Strait (Arnould et al. 2011) but also identical to the deep-diving oceanic sperm whale (see Figure 3.7, Table 3.3). Maria Island whales had a low diversity of cephalopods in their stomach contents with only ommastrephids that inhabit shelf and outer shelf waters around Tasmania, but no specifically neritic species such as *S. australis* or *O. moarum*. The ommastrephids in their stomachs did have comparable $\delta^{13}\text{C}$ values to their respective predators, suggesting that the squid were within their natural feeding habitat. However, in this study Ommastrephidae species were not differentiated based on their beak morphology. Of

the two common ommastrephid species found in these waters, both the shelf species *N. gouldi* and the oceanic species *T. filippovae* have previously been documented in two stranded pilot whales in Tasmanian waters. However, the former were a more numerically important prey item, along with other neritic cephalopods (Gales & Pemberton 1992).

In comparison to the Maria Island whales, the skin samples of Marion Bay whales were the most depleted in ^{13}C . They had similar $\delta^{13}\text{C}$ values to some slope demersal fish species that feed on benthic (e.g. morwong, pink ling) and pelagic prey (e.g. gemfish, blue grenadier) or shelf/slope species that prey on pelagic organisms from shelf waters (e.g. mirror dory) (Bulman & Koslow 2002). However, if whale skin $\delta^{13}\text{C}$ values are corrected to represent muscle, values approximated pelagic oceanic cephalopods (e.g. *H. atlantica*, *H. macrohista*, *C. veranyi*). Furthermore, only oceanic squid beaks were found in the stomachs of these whales. King Island whales, which had intermediate values were also similar to pelagic oceanic species but also shared comparable $\delta^{13}\text{C}$ values to some more neritic/demersal species.

Although all three whale stranding periods were similar between the two years (see Table 3.1), differences in the $\delta^{13}\text{C}$ values due to temporal variability at the base of the food web resulting from changes in temperature and productivity (McMahon et al. 2013) should not be discounted. Alternatively, a plausible explanation for the small differences exhibited in the $\delta^{13}\text{C}$ skin values may be due to differing foraging strategies. An offshore or pelagic foraging habitat for the Maria Island whales is unlikely as the data suggests a demersal or shelf foraging habitat. This is also supported by the slightly higher $\delta^{15}\text{N}$ values for the Maria Island whales which are a reflection of consistently richer ^{15}N baseline for both inshore and demersal habitats (McMahon et al. 2013). On the other hand, the King Island and Marion Bay whales were more likely to be feeding in pelagic slope or oceanic waters.

Variations in the diet of pilot whales due to seasonal, annual or geographical differences have been documented from previous studies on stomach content analysis (see Gannon et al. 1997, Santos et al. 2014). Stranded pilot whales from New Zealand waters have also shown evidence of a neritic versus oceanic diet for different whale strandings based on stomach content analysis, although it is unclear if this is due to unnatural

feeding prior to stranding (Beatson et al. 2007a, b, Beatson & O'Shea 2009). However, isotopic mixing models confirmed a coastal or demersal foraging habitat, as previously documented by stomach content analysis, for whales stranded in Northwest Iberia (Monteiro et al. 2015a). In contrast, whales stranded in Scotland had a more oceanic habitat and prey preference (Monteiro et al. 2015a).

Pilot whale diet (fish versus squid)

Dietary investigations based on stomach content analysis on long-finned pilot whales from various regions of the world suggest that they are predominantly, although not exclusively, a teuthophageous predator (Gannon et al. 1997, Santos & Haimovici 2001, De Pierrepont et al. 2005, Santos et al. 2014). Although pilot whales appear to be cephalopod specialist feeders, the variability in their diet based on temporal fluctuations and geographical regions suggests that they may be more generalist feeders, significantly influenced by the movement and abundance of prey resources (Santos et al. 2014). In the four strandings in this study where whale stomach contents were available, oceanic squids predominated. With the exception of ommastrephids, a common cephalopod family in all strandings, all species were ammoniacal squid primarily inhabiting the mesopelagic zone from slope to oceanic waters. However, common ommastrephids around Tasmania are known to inhabit shelf (*Nototodarus gouldi*) to oceanic waters (e.g. *Todarodes filippovae*). Furthermore, Octopoteuthidae may possibly be mesopelagic to demersal (Table 3.2). A number of other studies from various regions of the world, including Tasmania, have recognized both oceanic as well as neritic cephalopod species, including benthic octopods as significant prey (Gales & Pemberton 1992, Beatson et al. 2007a, b, Beatson & O'Shea 2009). This is in conjunction with a less important proportion of fish and invertebrates such as salps (e.g., Santos & Haimovici 2001, Spitz et al. 2011, Mèndez-Fernandez et al. 2012, Santos et al. 2014). In contrast, pilot whales from the mid-Atlantic have been documented as having a predominantly fish-based diet supplemented by squid (Overholtz & Waring 1991).

Whether a predominance of fish or squid has relative greater dietary importance is a central question in the foraging strategy of pilot whales and for other marine higher order predators (e.g. Cherel et al. 2008). Historically, almost all of the dietary information for whales, including pilot whales, has come from stomach content analysis. Although this method provides an important taxonomic framework for documenting

the predator-prey relationship, there are some caveats (Young et al. 2015a). In stranded whales there are well known inherent sources of bias due to stomach content analysis that only represents a recent snapshot of the predator's diet. Differential digestion of prey in addition to differing retention rates may significantly alter dietary estimates. Most dietary analysis is based on hard part analysis and although there is individual variation between and within predators the retention rates of most fish otoliths range from a few hours to a maximum of a few days. In contrast, cephalopod beaks that can get caught in the folds of the stomach lining may be retained from days to weeks or longer (Tollit et al. 2010, Xavier et al. 2011). This may lead to a comparative overestimation in the diet of cephalopods relative to fish. These biases are particularly relevant to studies on stranded whales since they may not have recently foraged in their natural habitat or may not even have been eating recently, skewing dietary estimations towards what is retained in the stomach the longest.

Stomach content analysis on two long-finned pilot whales from Tasmanian waters documented a primarily cephalopod diet supplemented by fish (Gales & Pemberton 1992). Conversely, fatty acid analysis on the blubber of stranded pilot whales from this region suggested a myctophid signature (Walters, 2005). However, an examination of $\delta^{13}\text{C}$ values for four myctophid species from this region revealed that only the myctophid *L. hectoris* could be a potential prey resource (see Table 3.3). Consumers feeding primarily on *L. hectoris* from the Tasmanian shelf break waters include jack mackerel, blue warehou and blue grenadier (Blaber & Bulman 1987, Bulman & Koslow 2002). While only the $\delta^{13}\text{C}$ values for blue grenadier paralleled the whales, the $\delta^{15}\text{N}$ values were significantly higher. However, since there may be differing discrimination factors for $\delta^{15}\text{N}$ between consumers and their prey the possibility of pilot whales also eating myctophids is not precluded. Nonetheless, the potential average discrimination factor between *L. hectoris* and all pilot whales would require a lower factor (1.1) than has previously been reported between skin values of closely related marine mammal species, such as the bottlenose dolphin, and their diet (Browning et al. 2014, Giménez et al. 2016). Furthermore, myctophids have not been documented as significant prey items in the stomach contents of pilot whales from this or other regions of the world. For example, there was evidence of ingestion of only two myctophids in pilot whales stranded in the western North Atlantic (Gannon et al. 1997).

An alternative explanation to the predominance of a potential myctophid diet in pilot whales may be secondary ingestion. Ommastrephids were a common prey item across all strandings. An examination of the diet of *T. filippovae* using stomach content analysis in addition to fatty acid analysis revealed a diet dominated by myctophids (Pethybridge et al. 2013). *T. filippovae* is a common slope/oceanic species in Tasmanian waters (Jackson et al. 2006). Likewise, the predominantly piscivorous portion of the diet of *N. gouldi*, a common ommastrephid inhabiting the continental shelf waters around Tasmania, was dominated by two mesopelagic fishes, including the myctophid *L. hectoris* (Pethybridge et al. 2012). Similarly, *Ommastrephes bartramii* in the central north Pacific was also found to have a diet dominated by myctophids (Watanabe et al. 2004) as well as the jumbo squid *Dosidicus gigas* in the central Gulf of California (Markaida et al. 2008). This corroboration of trophic data supports the proposal that myctophids are a significant prey for ommastrephids in outer shelf and oceanic waters (Rodhouse & Nigmatullin 1996). The predominance of ommastrephids in the diet of pilot whales in concert with their corresponding myctophid prey may explain the resulting fatty acid myctophid signature found by Walters (2005). This raises the question of a secondary trophic effect (see Cherel et al. 2008). Squid primarily have a protein metabolism, and therefore fats from their diet are dumped relatively unchanged into the digestive gland and excreted (Semmens 1998, Jackson & O'Dor 2001). If whole squid or digestive glands were used as a squid prey marker in fatty acid analysis, predators consuming squid would subsequently inherit the signature of the prey of the squid. This myctophid signature has also been found in the demersal onychoteuthid squid *Moroteuthis ingens* (Phillips et al. 2003). Even though *M. ingens* were not indicated in the diet of pilot whales, other oceanic squids from this region are likely to also consume myctophids, although this is still to be determined.

In this study, evidence suggests a clear preference for oceanic squid based on a comparison of $\delta^{13}\text{C}$ values of pilot whales with beaks from stomach contents. However, the range in $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ values suggests that they are also opportunistic. With the exception of *L. lorigera* and some medium sized ommastrephids, all other squid species represented in the stomachs from the four whale strandings displayed a similar $\delta^{15}\text{N}$ value to the whales or were more enriched in ^{15}N than any of the whale strandings, including their respective whale predators. Although Marion Bay whales consumed

squid with both similar and higher $\delta^{15}\text{N}$ values than themselves they also consumed a large number of *L. lorigera* which were the only squid from analyzed stomach contents that were consistently more depleted in ^{15}N than the whales from all strandings. A difference of 1.6 ‰ between the skin of Marion Bay whales and *L. lorigera* suggests a very low discrimination factor. Correspondingly, only the smaller *T. pellucida* and *M. ingens* from other potential squid resources from Tasmania were significantly lower than all the whales. However, recent experimental feeding studies of captive bottlenose dolphins suggests that while the commonly assumed trophic discrimination factor of 1 ‰ for ^{13}C is reasonable, a much lower trophic discrimination factor of 1.5 - 2 ‰ for ^{15}N is likely, rather than the more commonly assumed values of 2 - 5 ‰ (Browning et al. 2014, Giménez et al. 2016). In contrast, although the ommastrephids from the stomachs of Maria Island whales had similar ^{13}C values, the squid beaks were significantly more enriched in ^{15}N . If we are able to apply the turnover rate or retention time of ^{15}N reported for the skin of bottlenose dolphins (i.e. greater than 100 days, Giménez et al. 2016) we can assume that ommastrephids of this size were not their predominant food source in the months prior to stranding.

While the data from this study suggests that pilot whales have a preference for oceanic squid, it is also likely that they may be supplementing their diet with other organisms. The much higher $\delta^{15}\text{N}$ value of the primarily teuthophageous sperm whale foraging in similar waters (Evans & Hindell 2004a) suggests they are feeding at approximately one trophic level higher than the pilot whales. Alternatively, pilot whales may be consuming smaller-sized squid since $\delta^{15}\text{N}$ is positively related to cephalopod size (Cherel et al. 2009b, Chapter 2). However, there was no evidence for this even given the diversity of cephalopod species consumed (e.g. Marion Bay and Bicheno strandings). Over extended time periods smaller beaks may be more likely to be retained in the folds of the stomach lining (Tollit et al. 2010). The fact that skin $\delta^{15}\text{N}$ values for the whales were equivalent or lower than most beaks from their stomachs, as well as many potential squid resources, supports the idea that they may be also ingesting less ^{15}N enriched organisms simultaneously. This is irrespective of the discrimination factor between diet and whale skin possibly being lower than previously thought. For example, in the Bay of Biscay, in the Northeast Atlantic, pilot whales sometimes consumed large numbers of salps in addition to mostly small cephalopods and fish species (Spitz et al. 2011).

Gannon et al. (1997) documented the consumption of the small plankton-feeding fish Atlantic herring *Clupea harengus*, although there were relatively low numbers in comparison to cephalopods. However, given the strongly acidic stomachs of marine mammals, most fish hard parts may pass through the digestive system and be completely digested between one and three days after consumption (Tollit et al. 2010; Bowen & Iverson 2013). This is an inherent source of bias particularly for stranded whales as the timing and location of their recent foraging is unknown.

In general, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in this study are in agreement with that found for pilot whales by Davenport & Bax (2002). Like that found for many other studies on long-finned pilot whales, intrinsic factors revealed little variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for pilot whales. There is some support for differences due to foraging habitat (i.e. demersal/shelf versus slope/oceanic) although overall pilot whale isotopic values were fairly homogenous. This is the first study to compare actual isotopic values of pilot whale stomach contents to their respective predator. More of such comparisons are needed to provide definitive answers to the ongoing question of the relative importance of fish versus squid (or other prey) in the diet of marine mammals. Our isotopic data does not appear to support an exclusive diet of either cephalopods or myctophids. The pilot whales may exhibit a plastic foraging strategy, but largely teuthophageous, with a preference for oceanic squid (giving its strong myctophid fatty acid signature) and potentially supplementing their diet with other less enriched ^{15}N organisms. These less enriched ^{15}N organisms may be gelatinous and nearly impossible to detect in stomach contents of stranded whales. Furthermore, it is noteworthy that within the Tasmanian pelagic ecosystem, pilot whales had the lowest $\delta^{15}\text{N}$ value compared to most other marine mammal predators.

CHAPTER FOUR

WHO IS AT THE TOP? – ASSESSING THE PREDATOR-PREY RELATIONSHIP OF SPERM WHALES

Abstract

The ability to provide appropriate management and conservation of recovering populations of whales demands a comprehensive understanding of predator-prey relationships. Moreover, this is even more essential for sperm whales (*Physeter macrocephalus*) given their remote foraging habitats in the oceanic meso- and bathypelagic zone. This study investigated the diet and foraging range of sperm whales. Stable isotope analysis on skin samples was used to investigate the diet of sperm whales from five mass strandings that occurred from 2002 to 2005 in waters around the Island state of Tasmania, off southern Australia. The average skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sperm whales across all strandings ($n = 39$) were -17.1 ± 0.6 and 14.7 ± 0.8 ‰ respectively. Based on skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, sperm whales from different strandings appeared to show low variation in foraging range but were confirmed to be a higher order predator in this region. Since sperm whales are largely considered to be teuthophagous, isotopic analysis on a total of 313 squid beaks encompassing 10 different families from whale stomachs was undertaken. A comparison of $\delta^{13}\text{C}$ values of sperm whale skin and squid beaks from whale stomach contents to $\delta^{13}\text{C}$ values of squid species caught in Tasmanian waters suggests that the sperm whales had been foraging in an analogous marine ecosystem to that of waters around Tasmania. The $\delta^{15}\text{N}$ mean values of cephalopod beaks from sperm whale stomachs across all strandings ranged from 7.8 ± 1.2 ‰ (Ommastrephidae sp.) to 13.0 ± 0.7 ‰ (the colossal squid *Mesonychoteuthis hamiltoni*) with an overall difference of 5.2 ‰ equivalent to an estimated 1.5 - 2 trophic levels. Corrected $\delta^{15}\text{N}$ values of some squid species from whale stomachs had comparable values that often exceeded that previously recorded for the killer whale ($\delta^{15}\text{N}$ 15.2 ‰) from Tasmanian waters (e.g. *M. hamiltoni* 16.8 ± 0.7 ‰). Sperm whales exhibited intermediate $\delta^{15}\text{N}$ values relative to their prey. Their high trophic position is indicative of a $\delta^{15}\text{N}$ value based on consumption of cephalopod prey of varying trophic levels, although likely weighted towards smaller individuals (<400 mm DML). This study highlights that concurrent stable isotope analysis on both predator and prey can increase our understanding of the foraging range of sperm whales relative to the distribution of their prey.

INTRODUCTION

Tracing the trophic ecology of marine apex predators is essential to our understanding of ecosystem health and sustainability. The fundamental drivers as well as the scale of potential impacts on an ecosystem can be more readily determined by having a complete understanding of the predator-prey relationship (Ramos & González-Solís 2012). Large predators exert a significant influence on structuring the marine food web due to their direct impact on prey populations (Davis et al. 2007, Harvey et al. 2014). The energetic demands required by some of the larger marine mammals, such as cetaceans, have the potential to exert top-down effects on prey populations (Surma & Pitcher 2015). Therefore, understanding the feeding ecology of cetaceans is paramount for the management and conservation of recovering whale populations that were historically depleted from commercial whaling over the last two centuries (Baker & Clapham 2004, Surma & Pitcher 2015).

Sperm whales *Physeter macrocephalus*, the largest of all toothed whales, are members of the oceanic mesopelagic zone feeding predominantly on mesopelagic and bathypelagic organisms (Davis et al. 2007). The males and females segregate in terms of social organization and geographic distribution (Teloni et al. 2008). Mature females along with both immature males and females are usually distributed in social groups constrained to tropical and temperate waters between 40°N and 40°S (Whitehead et al. 1991, Teloni et al. 2008). As males mature they disperse poleward either individually or in small groups, only returning to warmer waters to breed (Mendes et al. 2007b). As individuals, these deep-diving oceanic foragers appear to be opportunistic predators but as a whole they appear to specialize on particularly abundant groups of prey (Evans & Hindell 2004a). Sperm whales, for the most part, appear to be teuthophageous, with dominant squid prey from the families Architeuthidae, Ommastrephidae, Octopoteuthidae, Histiotteuthidae, Ancistrocheiridae, Onychoteuthidae and Cranchiidae (Clarke 1980, Clarke & MacLeod 1980, Clarke & MacLeod 1982, Clarke et al. 1993, Santos et al. 2002, Evans & Hindell 2004a, André et al. 2007, Garibaldi & Podesto 2014, Harvey et al. 2014). Alternatively, they have been known to forage on fish, but to a much lesser degree (Clarke 1980, Martin & Clarke 1986, Santos et al. 2002). Due to their worldwide distribution and extreme large size, a conservative estimation of the annual consumption of squid by sperm whales may exceed 100 million tonnes, with between

12.5 and 24.2 million tonnes being consumed in the Antarctic Ocean alone (Santos et al. 2001, Whitehead 2002). Subsequently, understanding the trophic dynamics of sperm whales that forage at great depths also helps to elucidate the dynamics of deep-sea cephalopod assemblages (Harvey et al. 2014).

The logistics of determining trophic relationships between sperm whale and their prey *in situ* is extremely difficult due to their remote foraging habitats (Ruiz-Cooley et al. 2012). Stomach content analysis from stranded or commercially caught whales has largely been the predominant mode of investigation. Although this method has provided crucial information to our understanding of the predator-prey relationship of these apex predators, there are inherent biases in this single methodology. While stomach content analysis on commercially caught sperm whales had a bias towards adult animals, particularly males, single stranded animals may be biased toward unhealthy individuals (Evans and Hindell 2004a). In contrast, mass stranded animals are less likely to be affected by these biases (Evans & Hindell 2003b). Bogomolni et al. (2010) found that for single strandings in the Cape cod and Massachusetts area disease was the leading cause of death. However, around 92% of animals from mass strandings in the same area were healthy and most likely died as a result of stress or conditions related to the stranding itself. Furthermore, stomach content derived data is constrained by differential identification problems associated with the predator's prey. The combination of a rapid breakdown of gelatinous and soft-bodied animals but with a preferential retention of hard parts such as squid beaks, in conjunction with a rapid digestion of smaller organisms and those with fewer hard parts, may subsequently skew the resulting analysis in favor of those organisms represented by the least digestible parts (Santos et al. 2001, Young et al. 2015a).

Stable isotope analysis has been used extensively to understand the foraging ecology of a variety of marine mammals (see Newsome et al. 2010 for review) including sperm whales (Ruiz-Cooley et al. 2004, Marcoux et al. 2007, Mendes et al. 2007a, b, Ruiz-Cooley et al. 2012). The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios of a consumer provide an average time-integrated assimilated representation of an organism's diet on a broad scale rather than a dietary snapshot offered by stomach content analysis. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of various animal tissues reflect the averaged integrated diet of the consumer at varying time intervals, which are tissue and species dependent (de Niro & Epstein 1978, 1981,

Ruiz-Cooley et al. 2012). There are no documented turnover rates for sperm whale skin although Ruiz-Cooley et al. (2004) suggested the rate is likely greater than 72 d. A recent controlled feeding study on bottlenose dolphins *Tursiops truncatus* estimated a half-life of 24 ± 8 d and 48 ± 19 d for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, which translates to approximately 100 – 200 d for a near complete turnover rate of skin (95%) (Giménez et al. 2016). Since sperm whales are larger animals and have thicker skin than bottlenose dolphins the estimated time taken for a complete turnover or renewal of skin may even be longer.

$\delta^{13}\text{C}$ values have been mostly used in marine predators to differentiate between differing foraging habitats due to latitudinal and regional differences in $\delta^{13}\text{C}$ values at the base of the food chain along with small variations along the length of the food chain (Cherel & Hobson 2007). Determination of the foraging habitat is possible when the isotopic value of the consumer is compared with isotopically distinct latitudinal gradients in $\delta^{13}\text{C}$ values at the base of the food web in conjunction with the inshore/offshore and pelagic/demersal $\delta^{13}\text{C}$ gradients (Cherel & Hobson 2007). When examining tooth annuli of sperm whales, Mendes et al. (2007b) found a shift in $\delta^{13}\text{C}$ values denoting a male dispersion from the maternal pod that was also consistent with a poleward migration to more temperate oligotrophic waters. In contrast, $\delta^{15}\text{N}$ values have been used as a proxy for trophic position as well as an indicator of a predator-prey relationship (Post 2002). Predator-prey relationships are determined through the examination of the discrimination factor, which is the difference between the isotope value of the food of the consumer and the isotopic value of the consumer's tissue (Bond & Hobson 2012, Greer et al. 2015). There is predictable stepwise ^{15}N enrichment between the consumer and their prey for each increasing trophic level in the marine ecosystem (Kelly 2000). The average trophic discrimination factor between consumer and their food is ~ 3.4 ‰, but varies according to species or tissue examined (Post 2002). Marcoux et al. (2007) and Ruiz-Cooley et al. (2004) have found discrimination factors for ^{15}N between sperm whale and squid prey that fit well with the expected average trophic enrichment values for predator-prey relationships. Sperm whale diet based on isotopic values has also been shown to be spatially dependent (Marcoux et al. 2007). Isotopes have also further enhanced our understanding of sperm whale foraging

by determining the community assemblage of their cephalopod prey through isotopic analysis of squid beaks from stomach contents (Cherel et al. 2009a).

The purpose of this study was to undertake a study on the foraging ecology of sperm whales by performing an isotopic comparative analysis on the skin of animals that had mass stranded in Tasmanian waters off southern Australia. Additionally, since isotopes of the predator alone only give a broad scale view of foraging, sperm whale skin isotopic analysis in concert with isotopes of actual known and identified prey will give a more detailed picture of their foraging habits. Since Evans and Hindell (2004a) found that cephalopods predominated in the stomach contents of whales in mass strandings in Tasmanian waters, this study was focused on assessing the isotopic value of cephalopod beaks relative to the skin of the sperm whales to ascertain predator-prey relationships. Furthermore, since cephalopods are important trophic links for many marine mammals in the marine ecosystem another objective was to analyze the cephalopod community using the sperm whales as biological samplers.

METHODS

Sample collection

Sperm whale samples were obtained from archived material from mass strandings that occurred in northern and western coastal regions off Tasmania, Australia (i.e. Croppies Beach, 2002; Flinders Island, 2003; Ocean Beach, 2004; Strahan 2004 and Bakers Beach 2005). Ocean Beach and Strahan have virtually the same latitude and longitude, therefore only Ocean Beach is shown on the map but the two names are kept for distinguishing between strandings (Table 4.1, Figure 4.1). The archived samples were obtained using standardized mass stranding sampling protocol (Geraci & Lounsbury 2005) and held by the Department of Primary Industries, Parks, Water and Environment (DPIPWE). Whale skin samples of approximately 1 cm² were subsampled from frozen blocks of skin and blubber held in a -20° C freezer and originating from five mass strandings occurring in Tasmanian waters from 2002 to 2005. Dorsal, lateral or ventral samples were taken from a standardized body site just anterior to the dorsal fin. Each animal was sexed and measured for total length (m) by using a straight line from the tip of the rostrum to the deepest part of the notch in the tail fluke. They were assigned an age class (adult, subadult and juvenile/calf) based on length and maturity.

Cephalopod beaks from stomach contents were only collected for the Bakers Beach and Flinders Island mass strandings (see Figure 4.1). For comparative purposes and to enhance the overall picture of sperm whale diet, cephalopod beaks from a mass stranding in 2002 at Waterhouse Island were also used. All cephalopod beaks had been archived in 70 % ethanol and were subsequently identified to species level where possible (Clarke 1986, Xavier & Cherel 2009). Lower rostral lengths (LRL, nearest mm) were determined using digital calipers.

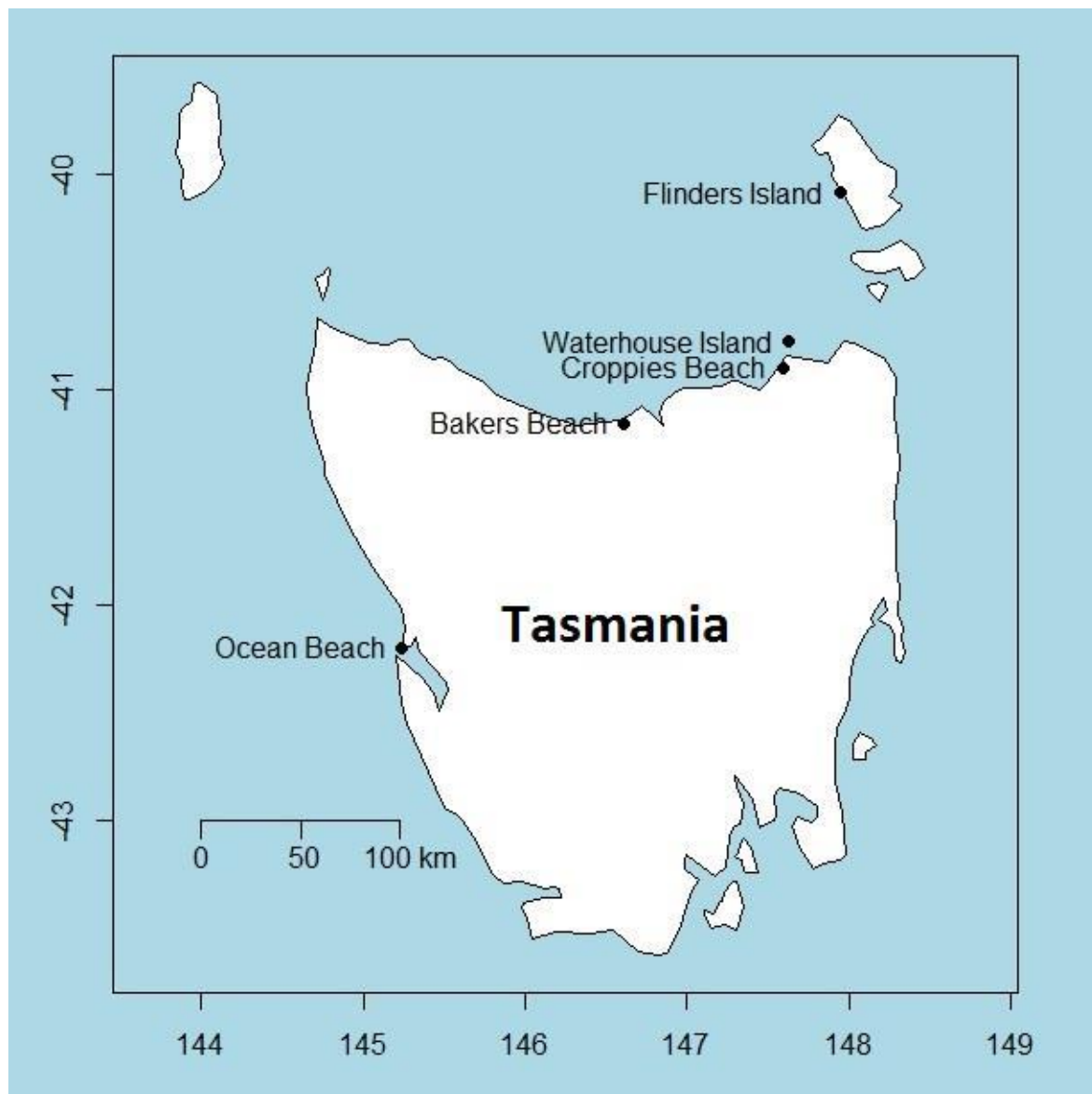


Figure 4.1. Map of Tasmania showing stranding sites of sperm whales (*Physeter macrocephalus*). Strahan has the same latitude and longitude as Ocean Beach.

Stable isotope analysis

In preparation for isotope analysis any remaining subcutaneous adipose tissue was removed with a scalpel from the whale skin subsamples. Samples were subsequently rinsed in distilled water. The frozen subsamples were then freeze dried, ground to a powder using a Wig-L-Bug® and delipidated in cyclohexane. The ground skin samples were delipidated using 3 ml of cyclohexane and left to stand overnight in small glass test tubes that were covered and put under a fume hood. Afterwards, samples were centrifuged and the supernatant removed with a pipette. The powdered whale skin was then subjected to two additional cyclohexane rinses with 1 - 2 hours between rinses. Next, samples were left to dry uncovered overnight under a fume hood.

All beaks were removed from ethanol and cleaned with distilled water. A small section of the wing tip in the direction of growth was cut and rinsed with distilled water. The tip of the cephalopod wing represents the most recent growth of the beak and subsequently the most recent somatic growth of the cephalopod (Cherel & Hobson 2005). Isotopic analysis was undertaken of whole wing tip samples or half wing tip samples for larger specimens. Where possible, 10 replicate individuals were processed for the size mode using LRL of each species within each stranding when stomach contents were retrieved. The relative abundance of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes was determined by a Finnigan Delta Plus Advantage stable isotope-ratio mass spectrometer at the University of Victoria, British Columbia, Canada. There was a 10 percent replication measurement in each isotopic sample run. The results of the isotopic analysis are presented in the usual delta notation relative to Vienna PD belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 (AIR) for $\delta^{15}\text{N}$. Replicate measurements of internal laboratory standards (DORM) indicated measurement errors of ± 0.1 and ± 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively.

Statistical analysis

Sperm whale skin

Since a standardized body site for sampling is sometimes difficult due to the orientation of the sperm whale once deceased, whale skin body site (i.e. dorsal, lateral and ventral) was used as a factor of interest in a repeated measures ANOVA for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Moreover, since the base of the food chain may fluctuate based on time and

space, ANOVA was used to ascertain any differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sperm whale skin due to stranding. MANOVA was used to determine if sex was a factor that contributed to the variation in average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Correlational analysis was also used to see if $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varied with whale length.

Stomach contents

Differences between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the same prey species from different strandings were also compared using ANOVA and t-tests to determine if whales from different strandings were foraging in similar locations. A comparison between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sperm whale skin relative to their diet across all strandings, as well as for the individual strandings and the respective squid beaks found in the stomachs of those animals, were assessed using ANOVA to ascertain if the contents found in the stomachs reflected their diet. Since ^{13}C is more enriched and ^{15}N more depleted in cephalopod beaks than corresponding muscle, values were corrected by -0.7‰ and $+3.8\text{‰}$ respectively to represent muscle (see Chapter 2). Samples were assessed for normality and those with less than three individuals were not used in analyses. As much as possible similar sized animals (as represented by LRL beak mode) were chosen for replication within a species. Post-hoc Tukey's multiple comparisons tests were used when a significant main effect was found.

RESULTS

Sperm whale skin isotopic analysis

Sperm whale skin ($n = 39$) was analyzed from five sperm whale strandings in Tasmanian waters (Croppies Beach, Flinders Island, Ocean Beach, Strahan and Bakers Beach) (see Table 4.1). Overall, the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of adults were $-17.1 \pm 0.6\text{‰}$ and $14.7 \pm 0.8\text{‰}$, respectively. Repeated measures ANOVA revealed no significant differences in the variation of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values between sperm whale dorsal, lateral and ventral skin sampling sites (both $p > 0.05$). MANOVA showed that sex had no effect on variation in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values ($p > 0.05$). Across all strandings whale length was not correlated with $\delta^{13}\text{C}$ values ($p > 0.05$) but weakly correlated with $\delta^{15}\text{N}$ values ($r = 0.4$, $n = 39$, $p = 0.007$). When examining males and females separately, females showed no correlation with $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (both $p > 0.05$). Males also did not correlate with $\delta^{13}\text{C}$ values but strongly correlated with $\delta^{15}\text{N}$ values ($r = 0.9$, $n = 13$, $p < 0.001$).

Variation in $\delta^{13}\text{C}$ values of sperm whale skin was dependent on stranding site (ANOVA $F_{3,31} = 3.9$, $p < 0.05$). Tukey's post-hoc multiple comparison tests separated the strandings into two groups. Bakers Beach had the lowest $\delta^{13}\text{C}$ values ($-18.1 \pm 2.3 \text{ ‰}$) and was significantly different from all other strandings ($p < 0.05$) (see Figure 4.2). However, when the Bakers Beach stranding was removed from the ANOVA ($F_{2,29} = 17.1$, $p < 0.0001$) due to an extreme outlier and a subsequent low number of individuals, multiple comparisons revealed that the $\delta^{13}\text{C}$ values of skin from Croppies Beach whales ($-16.6 \pm 0.5 \text{ ‰}$) were significantly higher than the Flinders Island ($-17.1 \pm 0.1 \text{ ‰}$) and Strahan ($-17.2 \pm 0.1 \text{ ‰}$) whales (both $p < 0.01$). The overall range in mean differences of $\delta^{13}\text{C}$ values between strandings was small when the Bakers Beach stranding was removed ($0.1 - 0.5 \text{ ‰}$). The Bakers Beach outlier (-20.05 ‰) had a much lower $\delta^{13}\text{C}$ value than the other two animals in the stranding ($-16.7 \pm 0.1 \text{ ‰}$) and increased the upper mean range difference to 1.4 ‰ . Ocean Beach whales and the calves from the Strahan stranding were excluded from the ANOVA due to insufficient numbers. Nevertheless, the average $\delta^{13}\text{C}$ values of their skin ($-16.7 \pm 0.6 \text{ ‰}$ and $-16.9 \pm 0.6 \text{ ‰}$ respectively) were within the restricted range of average $\delta^{13}\text{C}$ values recorded across all sperm whale strandings ($-16.7 \pm 0.3 \text{ ‰}$ to $-18.1 \pm 2.3 \text{ ‰}$, see Figure 4.2).

The average $\delta^{15}\text{N}$ values of whale skin between strandings also differed significantly ($F_{3,31} = 16.7$, $p < 0.0001$). Post-hoc multiple comparisons revealed that Bakers Beach whales had the lowest $\delta^{15}\text{N}$ values ($13.0 \pm 1.8 \text{ ‰}$) and differed from all other strandings, as did the Strahan stranding ($14.2 \pm 0.3 \text{ ‰}$) (all $p < 0.05$). Croppies Beach ($15.6 \pm 0.3 \text{ ‰}$) and Flinders Island ($14.9 \pm 0.4 \text{ ‰}$) whales had similar $\delta^{15}\text{N}$ values ($p > 0.05$) and were the most enriched in ^{15}N . Yet, when the Bakers Beach stranding was removed, due to reasons described above, $\delta^{15}\text{N}$ values of whale skin between strandings still differed (ANOVA, $F_{2,29} = 36.2$, $p < 0.0001$) but all strandings were significantly different than one another (Tukeys post-hoc multiple comparisons, all $p < 0.01$). Overall mean differences between strandings ranged up to approximately half a trophic level ($0.5 - 2.4 \text{ ‰}$) but without the Bakers Beach stranding the differences were smaller ($0.5 - 1.2 \text{ ‰}$). Calves from the Strahan stranding had the lowest $\delta^{15}\text{N}$ values ($12.9 \pm 0.9 \text{ ‰}$) relative to their own stranding ($14.2 \pm 0.3 \text{ ‰}$) and all other strandings (see Figure 4.2).

Stomach contents – isotopic analysis of cephalopod beaks

A total of 313 beaks were analysed from 10 squid families collected from the stomachs of sperm whales at three different strandings around Tasmania (Waterhouse Island, Flinders Island and Bakers Beach) from 2002 to 2005. The beaks used in the analysis were the predominant cephalopod species found across all strandings. Iconic species (small in number) such as *Architeuthis dux* (giant squid) and *Mesonychoteuthis hamiltoni* (colossal squid) were also included (see Table 4.2).

Table 4.1. Age class (based on length/maturity), sex, number of whales sampled (n), total length (m), skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C:N mass ratio values of sperm whales (*Physeter macrocephalus*) from five strandings around Tasmania, Australia (Croppies Beach, Flinders Island, Ocean Beach, Strahan and Bakers Beach). All values are means \pm SD.

Stranding (date)	Age category	Sex	n	Total length (m)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N (mass ratio)
Croppies Beach (November 2002)	Adults	F	6	11.1 \pm 0.7	-16.6 \pm 0.5	15.6 \pm 0.3	3.8 \pm 0.2
Flinders Island (November 2003)	Adult	M	9	11.8 \pm 0.5	-17.1 \pm 0.1	14.9 \pm 0.4	3.9 \pm 0.1
Ocean Beach (June 2004)	Adult	M	2	12.3 \pm 0.1	-16.7 \pm 0.1	14.8 \pm 0.2	3.8 \pm 0.0
Strahan (December 2004)	Adult	F	17	11.0 \pm 0.4	-17.5 \pm 0.1	14.2 \pm 0.3	4.0 \pm 0.1
	Calves	M	2	3.4 \pm 0.5	-16.9 \pm 0.6	12.9 \pm 0.9	3.2 \pm 0.2
Bakers Beach (December 2005)	Adult	F	3	11.0 \pm 0.6	-18.1 \pm 2.3	13.0 \pm 1.8	3.8 \pm 0.2
All combined	Adults		37	11.3 \pm 0.6	-17.1 \pm 0.7	14.6 \pm 0.9	3.9 \pm 0.1

Table 4.2. Beak lower rostral length (LRL), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (see Methods) and C:N mass ratios of cephalopod species retrieved from the stomach contents of sperm whales (*Physeter macrocephalus*) from three strandings around Tasmania, Australia (Waterhouse Island, Flinders Island and Bakers Beach). All values are means \pm SD.

Family	Species	N	LRL (mm)	$\delta^{13}\text{C}$ (‰)	Corrected $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Corrected $\delta^{15}\text{N}$ (‰)	C:N mass ratio
Waterhouse Is (November 2002)								
Enoploteuthidae	<i>Ancistocheirus lesueuri</i>	12	8.6 \pm 0.2	-16.5 \pm 0.3	-17.2 \pm 0.3	10.6 \pm 0.4	14.4 \pm 0.4	3.2 \pm 0.1
Octopoteuthidae	<i>Octopoteuthis</i> sp. (medium)	5	10.2 \pm 0.2	-16.8 \pm 0.3	-17.5 \pm 0.3	12.0 \pm 0.4	15.8 \pm 0.4	3.3 \pm 0.1
	<i>Octopoteuthis</i> sp. (large)	5	13.9 \pm 0.6	-17.0 \pm 0.1	-17.7 \pm 0.1	12.0 \pm 0.7	15.8 \pm 0.7	3.3 \pm 0.1
	<i>Taningia danae</i>	5	21.4 \pm 1.9	-17.2 \pm 0.3	-17.9 \pm 0.3	12.0 \pm 0.8	15.8 \pm 0.8	3.4 \pm 0.2
Onychoteuthidae	<i>Moroteuthis robsoni</i>	7	8.4 \pm 0.2	-16.6 \pm 0.2	-17.3 \pm 0.2	9.9 \pm 0.3	13.7 \pm 0.3	3.3 \pm 0.1
Cycloteuthidae	<i>Cycloteuthis akimushkini</i>	10	14.5 \pm 0.2	-17.3 \pm 0.3	-18.0 \pm 0.3	12.1 \pm 1.7	15.4 \pm 1.7	3.4 \pm 0.3
Lepidoteuthidae	<i>Pholidoteuthis boschmai</i>	11	9.4 \pm 0.1	-18.1 \pm 0.3	-18.8 \pm 0.3	9.4 \pm 0.7	13.2 \pm 0.7	3.3 \pm 0.1
Histioteuthidae	<i>Histioteuthis atlantica</i>	12	4.8 \pm 0.1	-17.1 \pm 0.3	-17.8 \pm 0.3	9.8 \pm 0.9	13.6 \pm 0.9	3.3 \pm 0.1
Chiroteuthidae	<i>Chiroteuthis veranyi</i>	12	7.3 \pm 0.1	-17.8 \pm 0.3	-18.5 \pm 0.3	11.6 \pm 1.6	15.4 \pm 1.6	3.4 \pm 0.2
Cranchiidae	<i>Taonius</i> sp. B (Voss)	3	10.4 \pm 0.4	-17.6 \pm 0.2	-18.3 \pm 0.2	12.3 \pm 0.3	16.1 \pm 0.3	3.3 \pm 0.2
	<i>Galiteuthis</i> sp. 3 (Imber)	12	7.5 \pm 0.2	-17.3 \pm 0.3	-18.0 \pm 0.3	10.1 \pm 1.3	13.9 \pm 1.3	3.4 \pm 0.1
	<i>Galiteuthis</i> stC sp. (Imber)	7	6.4 \pm 0.3	-17.2 \pm 0.3	-17.9 \pm 0.3	9.9 \pm 0.9	13.7 \pm 0.9	3.5 \pm 0.1
	<i>Mesonychoteuthis hamiltoni</i>	6	37.0 \pm 5.8	-17.4 \pm 0.9	-18.1 \pm 0.9	13.0 \pm 0.7	16.8 \pm 0.7	3.5 \pm 0.2
Flinders Island (November 2003)								
Enoploteuthidae	<i>Ancistocheirus lesueuri</i>	12	8.6 \pm 0.2	-16.7 \pm 0.4	-17.4 \pm 0.4	10.2 \pm 0.4	14.0 \pm 0.4	3.3 \pm 0.2
Octopoteuthidae	<i>Octopoteuthis</i> sp. (medium)	12	10.5 \pm 0.2	-16.9 \pm 0.3	-17.6 \pm 0.3	10.9 \pm 0.6	14.7 \pm 0.6	3.3 \pm 0.1
Onychoteuthidae	<i>Moroteuthis robsoni</i>	7	9.3 \pm 0.2	-16.8 \pm 0.3	-17.5 \pm 0.3	9.5 \pm 1.1	13.3 \pm 1.1	3.1 \pm 0.2
Lepidoteuthidae	<i>Lepidoteuthis grimaldi</i>	4	19.1 \pm 0.7	-17.1 \pm 0.4	-17.8 \pm 0.4	9.3 \pm 0.5	13.1 \pm 0.5	3.5 \pm 0.2
	<i>Pholidoteuthis boschmai</i>	7	9.3 \pm 0.2	-17.7 \pm 0.3	-18.4 \pm 0.3	9.5 \pm 1.0	13.3 \pm 1.0	3.4 \pm 0.2
Architeuthidae	<i>Architeuthis dux</i>	1	6.7	-16.5	-17.2	9.4 \pm 2.0	12.6	3.2
Histioteuthidae	<i>Histioteuthis atlantica</i>	12	4.7 \pm 0.2	-17.0 \pm 0.2	-17.7 \pm 0.2	10.4 \pm 0.7	14.2 \pm 0.7	3.3 \pm 0.1
	<i>Histioteuthis hoylei</i>	9	6.6 \pm 0.3	-17.1 \pm 0.1	-17.8 \pm 0.1	9.6 \pm 0.6	13.4 \pm 0.6	3.3 \pm 0.0
	<i>Histioteuthis miranda</i>	12	5.8 \pm 0.2	-18.0 \pm 0.2	-18.7 \pm 0.2	9.2 \pm 0.4	13.1 \pm 0.4	3.4 \pm 0.1

Ommastrephidae	Ommastrephidae sp.	2	14.2 ± 0.2	-17.6 ± 0.0	-18.3 ± 0.0	7.8 ± 1.2	11.6 ± 1.2	3.5 ± 0.0
Chiroteuthidae	<i>Chiroteuthis</i> sp. F (Imber)	8	5.6 ± 0.2	-17.5 ± 0.5	-18.2 ± 0.5	11.7 ± 2.2	15.5 ± 2.2	3.6 ± 0.2
Bakers Beach(December 2005)								
Enoploteuthidae	<i>Ancistocheirus lesueuri</i>	5	8.6 ± 0.2	-16.6 ± 0.1	-17.4 ± 0.1	10.0 ± 0.3	13.8 ± 0.3	3.3 ± 0.1
Octopoteuthidae	<i>Octopoteuthis</i> sp. (medium)	12	10.5 ± 0.3	-17.4 ± 0.6	-18.1 ± 0.6	10.4 ± 1.0	14.2 ± 1.0	3.2 ± 0.2
	<i>Octopoteuthis</i> sp. (large)	8	14.4 ± 0.3	-18.0 ± 0.6	-18.7 ± 0.6	10.5 ± 0.9	14.3 ± 0.9	3.3 ± 0.1
	<i>Taningia danae</i>	2	19.2 ± 0.3	-18.3 ± 0.0	-19.0 ± 0.0	11.0 ± 0.2	14.8 ± 0.2	3.4 ± 0.2
Onychoteuthidae	<i>Moroteuthis robsoni</i>	8	9.6 ± 0.3	-18.5 ± 0.6	-19.2 ± 0.6	8.4 ± 0.9	12.2 ± 0.9	3.3 ± 0.1
Lepidoteuthidae	<i>Pholidoteuthis boschmai</i>	6	9.4 ± 0.2	-18.4 ± 0.4	-19.1 ± 0.4	8.4 ± 0.6	12.2 ± 0.6	3.3 ± 0.2
Architeuthidae	<i>Architeuthis dux</i>	3	15.5 ± 3.0	-17.3 ± 1.1	-18.0 ± 1.1	9.4 ± 2.0	13.2 ± 2.0	3.4 ± 0.2
Histioteuthidae	<i>Histioteuthis atlantica</i>	24	5.2 ± 0.5	-17.9 ± 0.6	-18.6 ± 0.6	10.0 ± 0.9	13.8 ± 0.9	3.4 ± 0.1
	<i>Histioteuthis hoylei</i>	8	6.4 ± 0.2	-17.2 ± 0.2	-17.9 ± 0.2	9.8 ± 0.4	13.6 ± 0.4	3.4 ± 0.1
	<i>Histioteuthis miranda</i>	3	6.3 ± 0.1	-17.9 ± 0.2	-18.6 ± 0.2	9.7 ± 0.3	13.5 ± 0.3	3.4 ± 0.1
Ommastrephidae	Ommastrephidae sp.	5	13.9 ± 0.7	-17.2 ± 1.4	-18.9 ± 1.4	8.4 ± 0.8	12.2 ± 0.8	3.5 ± 0.1
Chiroteuthidae	<i>Chiroteuthis veranyi</i>	7	6.5 ± 0.2	-17.7 ± 0.6	-18.4 ± 0.5	11.0 ± 1.0	14.8 ± 1.0	3.5 ± 0.2
Cranchiidae	<i>Taonius</i> sp. B (Voss)	6	9.4 ± 0.3	-18.9 ± 0.8	-19.6 ± 0.8	11.3 ± 1.1	15.1 ± 1.1	3.4 ± 0.2
	<i>Teuthowenia pellucida</i>	12	5.2 ± 0.	-17.0 ± 0.3	-17.7 ± 0.3	11.5 ± 0.6	15.3 ± 0.6	3.4 ± 0.1
	<i>Megalocranchia</i> sp.	3	12.0 ± 0.3	-17.5 ± 0.4	-18.2 ± 0.4	10.7 ± 0.9	14.5 ± 0.9	3.7 ± 0.4
	<i>Galiteuthis</i> stC sp. (Imber)	6	6.6 ± 0.2	-17.4 ± 0.2	-18.1 ± 0.3	9.2 ± 0.9	13.0 ± 0.9	3.4 ± 0.2

Carbon isotopes

The mean $\delta^{13}\text{C}$ values of cephalopod beaks from whale stomach contents, across all strandings ranged from -18.9 ± 0.8 ‰ (*Taonius* sp. B (Voss)) to -16.5 ± 0.3 ‰ (*Ancistrocheirus lesueuri*) with an overall mean difference of 2.4 ‰. The mean overall differences in $\delta^{13}\text{C}$ values for beaks from the Waterhouse Island (1.3 ‰) and Flinders Island whales (1.6 ‰) were similar but lower than across all strandings. Conversely, the Bakers Beach overall mean difference in $\delta^{13}\text{C}$ values for beaks (2.2 ‰) was comparable to beak $\delta^{13}\text{C}$ values across all strandings (Table 4.2).

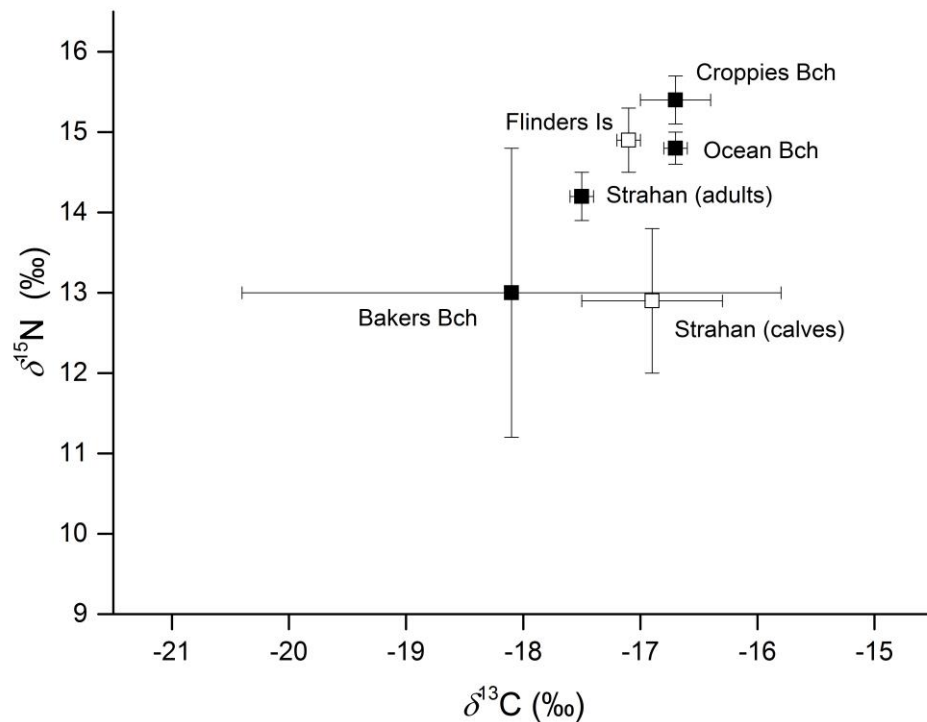


Figure 4.2. Mean (\pm SD) skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for sperm whale (*Physeter macrocephalus*) from strandings around Tasmania (Croppies Beach, Flinders Island, Ocean Beach, Strahan and Bakers Beach adults as well as Strahan calves). Open symbols are males, closed symbols are females.

Squid species represented in all three strandings included *A. lesueuri*, *Octopoteuthis* sp., *Moroteuthis robsoni*, *Pholidoteuthis boschmai* and *Histioteuthis atlantica*. When each species was compared individually between all three strandings, only *A. lesueuri* had similar $\delta^{13}\text{C}$ values to each other ($p > 0.05$). *Octopoteuthis* sp. (medium) ($F_{2,26} = 4.65$, $p < 0.02$), *M. robsoni* ($F_{2,19} = 44.33$, $p < 0.0001$), *P. boschmai* ($F_{2,21} = 6.78$, $p < 0.01$) and *H. atlantica* ($F_{2,45} = 16.08$, $p < 0.0001$) differed among strandings. Multiple comparisons revealed that *M. robsoni* and *H. atlantica* beaks from the Bakers Beach sperm whale stomachs had lower $\delta^{13}\text{C}$ values (-18.5 ± 0.6 ‰, -17.9 ± 0.6 ‰, respectively) than from both Waterhouse Island (-16.6 ± 0.2 ‰, -17.1 ± 0.3 ‰, respectively) and Flinders Island whale stomachs (-16.8 ± 0.3 ‰, -17.0 ± 0.2 ‰, respectively) (all $p < 0.05$). Furthermore, *Octopoteuthis* sp. (medium) and *P. boschmai* beaks from the Bakers Beach stranding also had significantly lower $\delta^{13}\text{C}$ values (-17.4 ± 0.6 ‰, -18.4 ± 0.4 ‰, respectively) than Flinders Island beaks (-16.9 ± 0.3 ‰, -17.7 ± 0.3 ‰, respectively) (all $p < 0.05$) but similar values to those from the Waterhouse Island stranding (all $p > 0.05$) (see Table 4.2).

Where cephalopod species only occurred in two of the three strandings, no significant differences in $\delta^{13}\text{C}$ values were found for *C. veranyi*, *Galiteuthis* stC sp. (Imber), *Histioteuthis hoylei*, *Histioteuthis miranda* or Ommastrephidae sp. (all $p > 0.05$). In contrast, both *Octopoteuthis* sp. (large) and *Taonius* sp. B (Voss) beaks from Bakers Beach sperm whale stomachs were more depleted in ^{13}C (-18.0 ± 0.6 ‰, -18.9 ± 0.8 ‰, respectively) than beaks from Waterhouse Island whales (-17.0 ± 0.1 ‰, -17.6 ± 0.2 ‰, respectively) (see Table 4.2).

Nitrogen isotopes

The mean $\delta^{15}\text{N}$ values of cephalopod beaks from sperm whale stomachs across all strandings ranged from 7.8 ± 1.2 ‰ (Ommastrephidae sp.) to 13.0 ± 0.7 ‰ (*Mesonychoteuthis hamiltoni*) with an overall difference of 5.2 ‰ or equivalent to an estimated 1.5 - 2 trophic levels. The overall mean difference in $\delta^{15}\text{N}$ values for beaks from Waterhouse Island (3.6 ‰), Flinders Island (3.9 ‰) and the Bakers Beach (3.1‰) strandings approximated a trophic level of one. When comparing the species common to all three strandings, only *A. lesueuri* ($F_{2,26} = 4.83$ $p < 0.05$), *M. robsoni* ($F_{2,19} = 6.39$, $p < 0.01$), *Octopoteuthis* sp (medium) ($F_{2,26} = 8.8$, $p < 0.01$) and *P. boschmai* ($F_{2,21} = 3.95$, $p < 0.05$) differed significantly between strandings. Although there was an

overall significant difference for *P. boschmai*, multiple comparisons tests showed the difference was marginal (all $p = 0.05$). However, the $\delta^{15}\text{N}$ values for *A. lesueuri* ($10.0 \pm 0.3 \pm \text{‰}$), *M. robsoni* ($8.4 \pm 0.9 \text{ ‰}$) and *Octopoteuthis* sp. (medium) ($10.4 \pm 1.0 \text{ ‰}$) from Bakers Beach were in all cases similar to the values of the Flinders Island stranding (all $p > 0.05$) but significantly lower than those of Waterhouse Island (10.6 ± 0.4 , 9.9 ± 0.3 , 12.0 ± 0.4 respectively, all $p < 0.05$). Similarly, *Octopoteuthis* sp. beaks (large) $\delta^{15}\text{N}$ values from Bakers Beach ($10.5 \pm 0.9 \text{ ‰}$) were significantly lower than beaks from the Waterhouse stranding ($12.0 \pm 0.7 \text{ ‰}$) ($F_{1,11} = 10.3$, $p = 0.008$). All other beaks of the same cephalopod species did not differ in $\delta^{15}\text{N}$ values between strandings (all $p > 0.05$) (see Table 4.2).

Isotopic comparison of sperm whale skin and squid beaks from stomach contents

The $\delta^{13}\text{C}$ values of sperm whale skin and the corrected $\delta^{13}\text{C}$ values of squid beaks from the whale stomachs differed significantly when combined and compared across all strandings ($F_{24,357} = 22.63$, $p < 0.0001$). Species were graphed according to increasing $\delta^{13}\text{C}$ values (Figure 4.3) and compared to three species caught *in situ* including *Histioteuthis atlantica* and the neritic *Sepioteuthis australis* captured in Tasmanian waters as well as the endemic Southern Ocean species, *Galiteuthis glacialis*. Based on $\delta^{13}\text{C}$ values of all species, Tukey's post-hoc multiple comparison tests segregated the squid and sperm whales into seven groups where there was a graduating and largely overlapping enrichment in ^{13}C from *G. glacialis* to the adult sperm whales which were most enriched in ^{13}C . The values of *H. atlantica* beaks caught in Tasmanian waters did not differ from those extracted from the sperm whale stomachs ($p > 0.05$). Although there was overlap in $\delta^{13}\text{C}$ values between *S. australis* and many squid species from the sperm whale stomachs they differed from *G. glacialis*, *C. veranyi*, *H. atlantica*, *H. miranda*, *Octopoteuthis* sp. (large), *P. boschmai* and *Taonius* sp. B (Voss) ($p < 0.01$). *G. glacialis* caught in Southern Ocean waters, on the other hand, differed from all other squid species as well as the sperm whales ($p < 0.0001$) (see Figure 4.3).

$\delta^{15}\text{N}$ values of combined sperm whales and squid beaks (corrected values) across all strandings segregated ($F_{21,334} = 15.74$, $p < 0.0001$) into nine overlapping groups. Post-hoc Tukey's multiple comparison tests showed that sperm whales had intermediate

values and were significantly different from only four squid species that were more impoverished in ^{15}N (i.e. Ommastrephidae sp., *P. boschmai*, *A. dux* and *M. robsoni*) and from *M. hamiltoni* which was the most enriched in ^{15}N of all species. *M. hamiltoni* was not significantly different than *Chiroteuthis* sp. F (Imber), *C. veranyi*, *C. akimushkini*, *T. pellucida*, *T. danae* and *Taonius* sp. B (Imber) ($p > 0.05$) (see Figure 4.4)

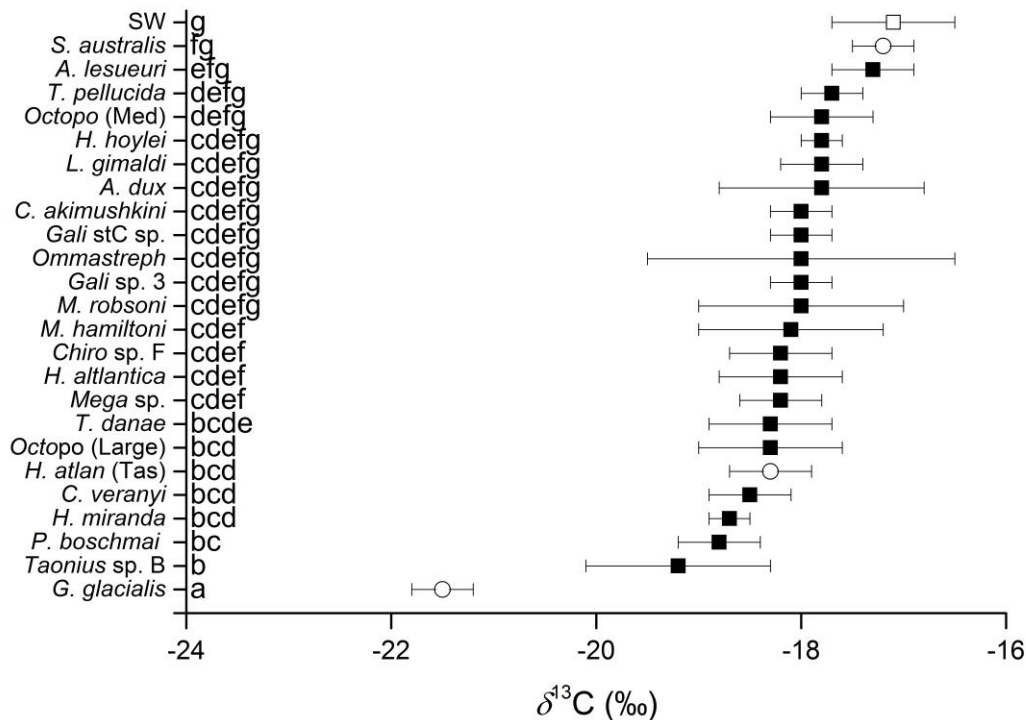


Figure 4.3. Mean (\pm SD) $\delta^{13}\text{C}$ values of skin from all sperm whale (*Physeter macrocephalus*) strandings (SW open square symbol; Croppies Beach, Flinders Island, Ocean Beach, Strahan and Bakers Beach) in comparison to all cephalopod corrected beak values (see Methods) from stomach contents. Open circle symbols refer to a comparison species (*G. glacialis*) from Maquarie Island as well as two species (*H. atlantica* and *S. australis*) from Tasmanian waters. Lower case letters refer to groups according to Tukey's post-hoc tests. *Taonius* sp. B, *Taonius* sp. B (Voss); *H. atlan* (Tas), *H. atlantica* (Tasmania); *Octopo* (Large), *Octopoteuthis* sp. (Large); *Mega* sp., *Megalocranchia* sp.; *Chiro* sp. F, *Chiroteuthis* sp. F (Imber); *Gali* sp. 3, *Galiteuthis* sp. 3 (Imber); *Ommastreph*, *Ommastrephidae* sp.; *Gali stC* sp., *Galiteuthis stC* sp. (Imber); *Octopo* (Med), *Octopoteuthis* sp. (medium).

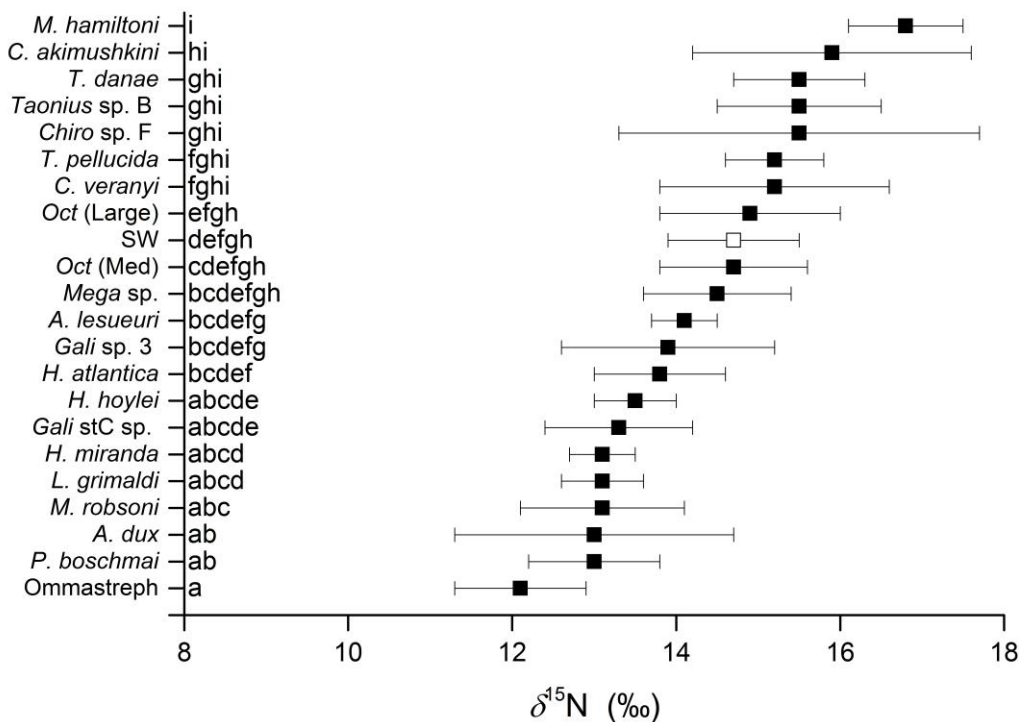


Figure 4.4. Mean (\pm SD) $\delta^{15}\text{N}$ values of skin from all sperm whale (*Physeter macrocephalus*) strandings (SW open square symbol; Croppies Beach, Flinders Island, Ocean Beach, Strahan and Bakers Beach) in comparison to all cephalopod corrected beak values (see Methods) from stomach contents. Lower case letters refer to groups according to Tukey's post-hoc tests. Ommastreph, Ommastrephidae sp.; *Gali* stC sp., *Galiteuthis* stC sp. (Imber); *Gali* sp. 3, *Galiteuthis* sp. 3 (Imber); *Mega* sp., *Megalocranchia* sp.; *Octopo* (Med), *Octopoteuthis* sp. (medium) *Octopo* (Large), *Octopoteuthis* sp. (Large); *Chiro* sp. F, *Chiroteuthis* sp. F (Imber); *Taonius* sp. B, *Taonius* sp. B (Voss).

A significant difference was found when comparing the $\delta^{13}\text{C}$ values between sperm whale skin of just the Flinders Island stranding and the respective beaks from their stomachs ($F_{9,82} = 25.0$, $p < 0.0001$). Tukeys post-hoc multiple comparisons test showed that the skin had similar values to the beaks of *M. robsoni* and *A. lesueuri* (both $p > 0.05$) but was significantly more enriched in ^{13}C than the beaks of *Chiroteuthis* sp. F (Imber), *H. atlantica*, *H. hoylei*, *H. miranda*, *L. grimaldi* and *Octopoteuthis* sp. LRL mm (all $p =$ or < 0.01). The $\delta^{15}\text{N}$ mean values of the sperm whale skin also differed from the cephalopod beaks found in their stomachs ($F_{9,82} = 8.033$, $p < 0.0001$). Multiple comparisons tests showed that the sperm whales had similar values to *Chiroteuthis* sp. F (Imber),

Octopoteuthis sp. (medium), *H. atlantica* and *A. lesueuri* (all $p > 0.05$). However, the whales were significantly more enriched in ^{15}N than *H. hoylei*, *P. boschmai*, *M. robsoni*, *L. grimaldi* and *H. miranda* (all $p < 0.03$) (see Figure 4.5).

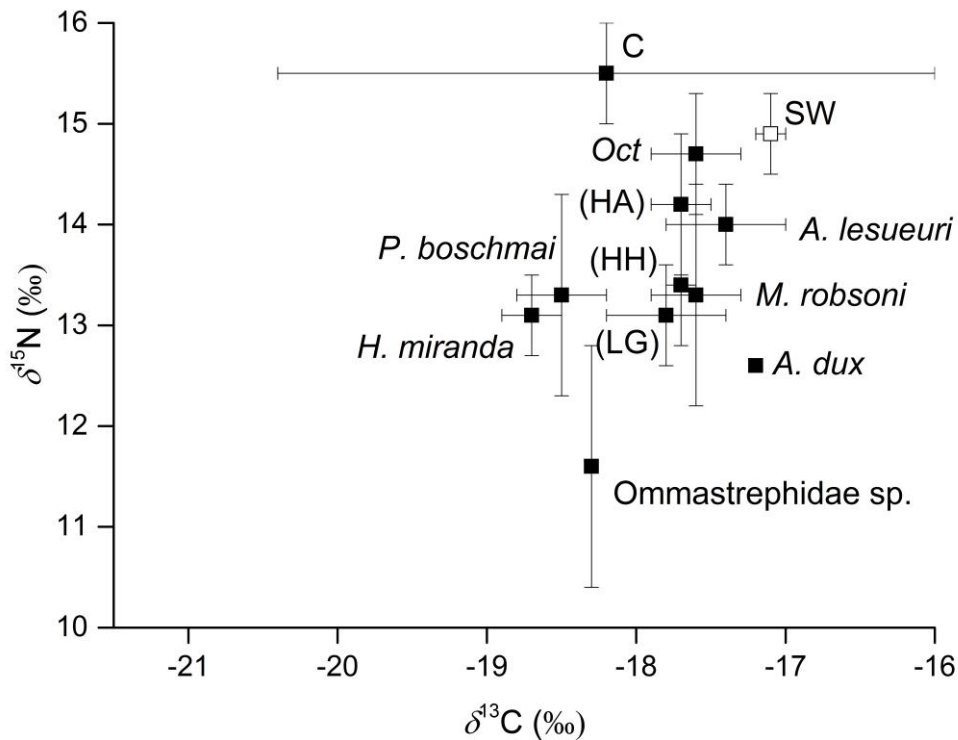


Figure 4.5. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of skin for sperm whale (*Physeter macrocephalus*) (SW, open square symbol) from the Flinders Is. stranding compared to corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (see Methods) of squid beaks from their stomach contents. C, *Chroteuthis* sp. F (Imber); Oct, *Octopoteuthis* sp. (medium); HA, *Histiotteuthis atlantica*; HH, *Histiotteuthis hoylei*; LG, *Lepidoteuthis grimaldi*.

The distribution across $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the skin from the Bakers Beach whales and the corrected values of cephalopod beaks from their stomachs significantly differed ($\chi^2_{16} = 60.97$, $p < 0.0001$; $\chi^2_{16} = 67.75$, $p < 0.0001$, respectively). However, multiple comparisons tests showed that the sperm whales did not differ from any of the cephalopod species for either isotope (all $p > 0.05$). This should be treated with caution due to the low number of whales for the Bakers Beach stranding ($n=3$, all female) and also the larger standard deviation for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (2.3 and 1.8 respectively)

due to the influence of an outlier ($\delta^{13}\text{C}$ -20.8 ‰, $\delta^{15}\text{N}$ -11.05) which was ~ 4 and 3 ‰ less enriched than the other 2 whales from the stranding in ^{13}C and ^{15}N respectively (see Figure 4.6)

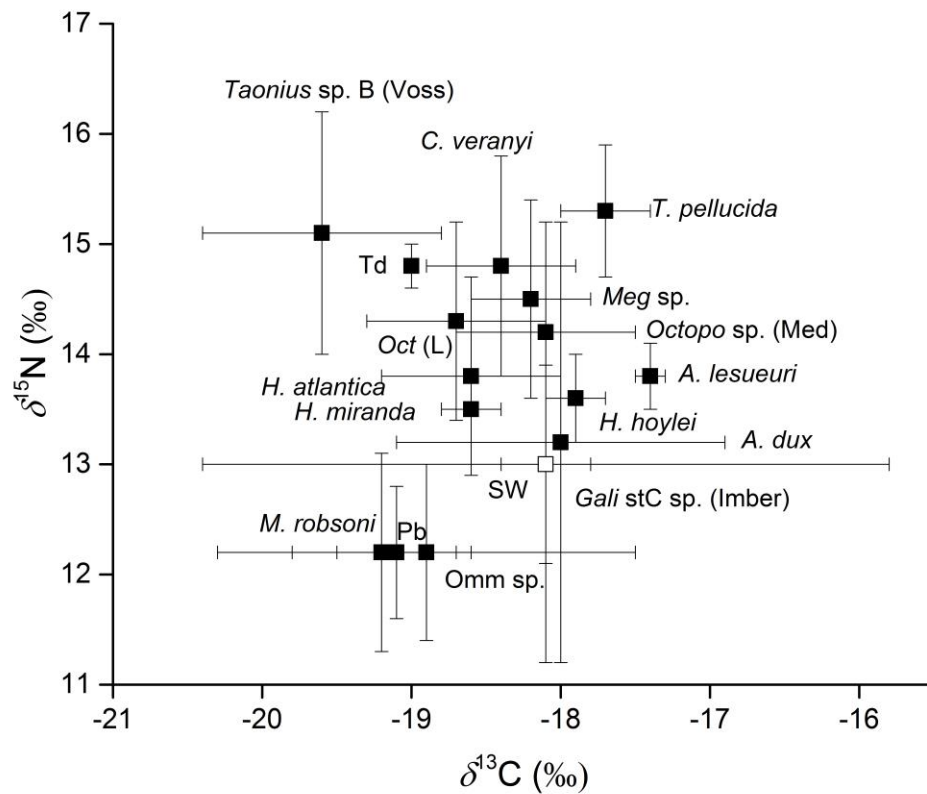


Figure 4.6. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of skin for sperm whale (*Physeter macrocephalus*) (SW, open square symbol) from the Bakers Beach stranding compared to corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (see Methods) of squid beaks from their stomach contents. Td, *Taningia danae*; Pb, *Pholidoteuthis boschmai*; Oct (L), *Octopo* sp. (Med), *Octopoteuthis* sp. (medium); *Octopoteuthis* sp. (large); Omm sp., Ommastrephidae sp.; Meg sp., *Megalochochanchia* sp.

DISCUSSION

Isotopic analysis on sperm whales from Tasmanian waters revealed that their foraging habits place them at a higher trophic level than most other cetaceans in this region, with the one exception being the killer whale ($\delta^{15}\text{N}$ 15.2 ‰) (Davenport & Bax 2002). The $\delta^{15}\text{N}$ values of the southern right whale dolphin *Lissodelphis peronei* (10.6 ‰), long-

finned pilot whale *Globicephala melas edwardii* (10.7 ‰), common dolphin *Delphinus delphis* (13.3 ‰) and gray's beaked whale *Mesoplodon grayi* (12.9 ‰) (see Davenport & Bax 2002) were all less than that observed for the sperm whales in this study (14.6 ‰). Some of the sperm whale prey in the present study had corrected $\delta^{15}\text{N}$ values comparable to and sometimes exceeding that of the killer whale *Orcinus orca*. Other studies have also found that some cephalopod species such as *M. hamiltoni* and *T. danae* are close to the top (i.e. top predator) in their ecosystems (Cherel & Hobson 2005, Cherel et al. 2009). Although sperm whales have a similar $\delta^{13}\text{C}$ value to long-finned pilot whales that have stranded in this region they are approximately one trophic level higher (Chapter 3). The high trophic position is likely indicative of a $\delta^{15}\text{N}$ value based on consumption of cephalopod prey of varying trophic levels, although likely weighted towards smaller individuals (< 400 mm DML) while the pilot whales conceivably feed on a more catholic diet of fish and squid or other organisms (Chapter 3).

Isotopic variation of sperm whale skin

Although there were small significant stranding effects on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of skin from sperm whales stranded in various regions around Tasmania, it is likely that none of the variation in this study was driven by differences in the skin anatomical sampling site between strandings (Williams et al. 2008). Additionally, since social structure is an important component of sperm whale populations with females and immatures living in long-term social units (Cristal & Whitehead 2001, Whitehead & Rendell 2004), it may be assumed that members of the same groups would have similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, resulting from traveling and foraging together (e.g. Marcoux et al. 2007). However, strandings may include casual acquaintances that are temporary associates rather than members of the stable social group (Cristal et al. 1998) and have subsequently foraged in isotopically different areas, resulting in increased isotopic variation within and between strandings. This may explain the female outlier in the Bakers Beach stranding that had lower isotopic values than the other females in that stranding. It is likely that the small variations found in the skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of this study may be a reflection of the fluctuations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at the base of the food chain rather than a change in diet.

The small variations seen in this study may be a response to latitudinal and regional fluctuations in ecosystem $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Latitude and region have been shown to

affect skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in sperm whales from the Gulf of Mexico and Gulf of California, most likely due to heterogeneity in baseline isotopic values that are subsequently transferred to consumers (Ruiz-Cooley et al. 2012). The overall mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in this study (-17.0 and 14.8 ‰, respectively) were closer to the values found for sperm whales from the Gulf of Mexico (-16.5 and 12.2 ‰, respectively, Ruiz-Cooley et al. 2012) and the Galapagos (~ -17.0 and 13.9 ‰, respectively, Marcoux et al. 2007). Sperm whale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were higher from the Gulf of California (-13.8 and 19.6 , respectively, Ruiz-Cooley et al. 2004) and Chile (~ -14.8 and 21.1 ‰, respectively, Marcoux et al. 2007). It is proposed that these contrasting isotopes are likely due to latitudinal and spatial differences in the dominant biochemical cycling within those waters (Ruiz-Cooley et al. 2012). The high CO_2 in waters in colder latitudes and regions results in a greater expression of photosynthetic CO_2 due to fractionation, producing a lower baseline $\delta^{13}\text{C}$ value. The reverse is true of waters in warmer latitudes (Newsome et al. 2010). Furthermore, in areas of high productivity and upwelling, waters can become suboxic to anoxic resulting in denitrification. Subsequently, an enriched residual nitrite pool is left due to ^{14}N enriched nitrite being preferentially removed which then leads to a higher ^{15}N baseline (Newsome et al. 2010). The Gulf of California and Chile are characterized by elevated primary productivity (Santamaría del Angel et al. 1994, Rendell et al. 2004) compared to the waters around Galapagos and the Gulf of Mexico (Palacios 2002, Wawrik & Paul 2004). Similarly, the waters of southern Australia and Tasmania are relatively oligotrophic (Harris et al. 1991, Prince 2001) which may help explain the comparable isotope values of sperm whales documented in this study to that of the Galapagos and the Gulf of Mexico.

Isotopic variance between strandings may also be attributed to seasonal or annual changes in the baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The variation between stranding $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in this study is confounded by temporal factors due to each stranding occurring in a different year. In the present study the overall range in mean differences between the strandings/years in $\delta^{13}\text{C}$ (0.1 - 1.4 ‰ or 0.1 - 0.5 ‰ with outlier removed) and $\delta^{15}\text{N}$ values (0.5 - 2.4 ‰ or 0.5 - 1.2 ‰ with outlier removed) were relatively small. Ruiz-Cooley et al. (2004, 2012) documented comparable values between years in sperm whales in the Gulf of Mexico and Gulf of California, suggesting that the whales maintained a similar diet temporally within their overall foraging area. Conversely,

Galapagos and Chilean sperm whales displayed larger variation in skin $\delta^{15}\text{N}$ values collected on various yearly scales, likely reflecting changes in $\delta^{15}\text{N}$ values at the base of the food chain or in foraging behaviour resulting from prey dynamics (Marcoux et al. 2007).

The calves and adults from the Strahan stranding exhibited similar $\delta^{13}\text{C}$ values but a surprising dissimilarity in $\delta^{15}\text{N}$ values with the calves being at least a half trophic level below the adults. A strongly held hypothesis suggests that since lactating marine mammals catabolize their tissues to produce milk, offspring should follow a pattern of trophic enrichment relative to their mothers (Newsome et al. 2010). However, the evidence is mixed. Although a recent study reported comparable values between adult and juvenile long-finned pilot whales (Chapter 3) the $\delta^{15}\text{N}$ values of juveniles or smaller individuals of bottlenose dolphins, including independently feeding juveniles were more enriched in ^{15}N compared to adults (e.g. Browning et al. 2014, Monteiro et al. 2015a, Fontaine et al. 2015). However, the $\delta^{15}\text{N}$ values between mother and offspring are tissue and species specific. There is often a lower mother-offspring discrimination factor when comparing tissues other than the mother's milk to the offspring (Cherel et al. 2015). Valenzuela et al. (2010) found very small $\delta^{15}\text{N}$ differences (+ 0.5 ‰) when skin was compared between mother-calf pairs. However, this discrimination value may increase if differences were compared between the actual mother's milk and the calf tissue (see Cherel et al. 2015). There may correspondingly be a lower $\delta^{15}\text{N}$ mother – calf discrimination value in cetaceans due to a possible lower overall discrimination factor (~ 1.5 ‰) between cetaceans and their prey, as was documented for captive bottlenose dolphins (Browning et al 2014, Giménez et al. 2016).

This study supports other studies that have found that diet changes with size (Evans & Hindell 2004a) at least for males. Mendes et al. (2007b) found an increase in $\delta^{15}\text{N}$ values in sperm whale teeth with age, and presumably size. Similarly, Borrell et al (2013a) also found an increasing trend in $\delta^{15}\text{N}$ values of teeth with age in male sperm whales from Denmark although one immature male and all female sperm whales from NW Spain exhibited a decreasing trend. The differences were explained in terms of greater diving prowess of males as they matured leading to capture of larger prey whereas females may reduce their diving depth due to reproductive activity. However, the opposing

trend of lower $\delta^{15}\text{N}$ values in the calves relative to the adult females from the Strahan stranding in this study is unclear. The number of calves in this study was small ($n = 2$) and an analysis of a larger number of juveniles and calves might increase our understanding on this issue.

Stomach contents

Overall, the cephalopod species represented by the beaks from stomach contents in this study encompassed a fairly restricted range in $\delta^{13}\text{C}$ values both within and between species that implies that they occupy closely related and overlapping habitats in the mesopelagic to bathyl ecosystems. This is likely reiterated by the lack of any clear pattern in the $\delta^{13}\text{C}$ values between beaks of the same species from different strandings. The exception being a few squid species from Bakers Beach that were more depleted in ^{13}C , most likely a result of individual differences in foraging exhibited in this group of females. The larger variation for the Ommastrephidae sp. is likely due to the representation of more than one species with possible differences in habitat. Occupancy of similar habitats by the whales was further confirmed by the isotopic position of the three squid species used in this study which were caught *in situ*, relative to those from whale stomach contents. *G. glacialis*, which was caught around the subantarctic Macquarie island, was the most depleted in ^{13}C and segregated from all other squid. This is possibly a result of higher latitudes exhibiting lower $\delta^{13}\text{C}$ values at the base of the food chain (Cherel & Hobson 2007). Conversely, the neritic squid *S. australis* caught in Tasmanian waters was the most enriched in ^{13}C of all the squid, similar to the $\delta^{13}\text{C}$ values for the sperm whale and other deepwater squid. This can be explained in terms of inshore/offshore and pelagic/demersal isotopic gradients whereby inshore and deepwater demersal isotopic baselines are more enriched in ^{13}C (Cherel & Hobson 2007). Furthermore, the $\delta^{13}\text{C}$ values of *H. atlantica* previously caught in Tasmanian waters did not differ from $\delta^{13}\text{C}$ values of *H. atlantica* beaks from the sperm whale stomachs (despite the beaks of *H. atlantica* being of different sizes). Additionally, the parallel $\delta^{13}\text{C}$ values of sperm whales and pilot whales (Chapter 3) stranded around Tasmania both share overlapping $\delta^{13}\text{C}$ values for a number of squid prey species found in their stomachs. The majority of the squid species found in the sperm whale stomachs (with perhaps the exception of *M. hamiltoni* and *Galiteuthis* sp. (Imber), and *H. hoylei*) have also been captured in deepwater trawls in Tasmanian coastal waters (G.

Jackson, personal communication). Together this data suggests that the beaks probably represent cephalopods inhabiting analogous marine ecosystems in the vicinity of Tasmania, or at least similar latitude. Sperm whale movement based on marking programs around Australia has shown that sperm whales move longitudinally from the eastern Indian Ocean across waters south of Australia and over to New Zealand (Brown 1981 cited in Evans & Hindell 2004a).

The small graduating increase in $\delta^{13}\text{C}$ values observed for the cephalopod species in this study may be attributed to horizontal/vertical distributions in habitat, whereby more pelagic species are ^{13}C depleted compared to more demersal species (see Cherel et al. 2009a). Nevertheless, the complete distribution and habitat of many deepwater oceanic cephalopods is unknown (Hoving et al. 2014) and often inferred. All cephalopod species represented in the sperm whale stomachs of this study most likely inhabited the meso-pelagic to bathypelagic zone. Moreover, all species were ammoniacal, with the exception of Ommastrephidae sp. from the Flinders Island and Bakers Beach strandings.

Overlapping $\delta^{13}\text{C}$ values of the colossal squid *M. hamiltoni*, which is often recorded in Antarctic waters, with other squid species from Tasmanian waters suggests that this species may have a wide distribution (Remeslo et al. 2015). The colossal squid is thought to predominantly inhabit Southern Ocean waters, distributed from the Antarctic Convergence to 70°S, and possibly penetrating northward to 40°S (Remeslo et al. 2015). The large variation in $\delta^{13}\text{C}$ values exhibited by the colossal squid may represent a continuum from subtropical to cooler southern waters (Cherel & Hobson 2005). Evans & Hindell (2004a) recorded Antarctic species from stomachs of sperm whales stranded in Tasmanian waters. It was proposed that either female and younger male sperm whales forage at lower latitudes than previously thought, or that the distribution of prey species is wider than previously known.

Sperm whales stranded in Tasmanian waters foraged on cephalopods spanning a continuum of two trophic levels. This is similar or slightly larger than that observed for cephalopod beaks from sperm whale stomachs stranded in the North Atlantic (Cherel et al. 2009a). The relatively narrow range in $\delta^{13}\text{C}$ values for the beaks permitted a comparison of the trophic position of species based on their $\delta^{15}\text{N}$ values. The graduating enrichment in ^{15}N between the squid species, from the lowest (Ommastrephidae sp.) to

the highest $\delta^{15}\text{N}$ values (the colossal squid) is likely due to a trophic continuum. The lower part of the trophic continuum includes species of ommastrephids (Pethybridge et al. 2012) as well as *Moroteuthis ingens* that feed on euphausiids and small fish like myctophids (Jackson et al. 1998, Philips et al. 2003) to the upper part of the continuum which includes the colossal squid which is expected to feed on larger fish and squid (Cherel & Hobson 2005). A similar trophic continuum was described for the Kerguelen cephalopod community (Cherel & Hobson 2005). *M. hamiltoni* was also reported as the highest predator in the Kerguelen cephalopod community (Cherel & Hobson, 2005). However, in the present study *M. hamiltoni* also overlaps with *C. akimushkini*, *T. danae* and four other cranchiids (*Taonius* sp. B (Voss), *Chroteuthis* sp. F (Imber), *T. pellucida* and *C. veranyi*). Two cranchiids, *Chroteuthis calyx* and *M. hamiltoni* have been reported to be slow moving rather than having an active lifestyle (Rosa & Seibel, 2010, Burford et al. 2015). The slow-moving sit-and-wait mastigoteuthid predator, *Idioteuthis cordiformis*, has also been shown to occupy a high trophic position in Tasmanian waters ($\delta^{15}\text{N}$, 15.8 ‰) (Chapter 2). Braid and Bolstad (2014) likewise obtained high $\delta^{15}\text{N}$ values for *I. cordiformis* and reported that based on DNA barcoding a large pelagic birdbeak dogfish and snapper were eaten by this species in New Zealand waters. The cranchiids, like the mastigoteuthid *I. cordiformis*, are also likely to be ambush-predator or sit-and-wait predators that may use suckers or hooks on their arms and tentacles to catch unsuspecting prey (Hoving et al. 2014) thus enabling them to catch larger prey higher up the food chain. This may explain why more fragile species such as *T. pellucida* have higher $\delta^{15}\text{N}$ values than some larger more robust cephalopod species such as *A. dux*.

Whale skin and cephalopod beaks

Sperm whale skin had the highest $\delta^{13}\text{C}$ values measured in this study but shared a similar trophic position to some of their prey, with some corrected $\delta^{15}\text{N}$ values exceeding that of the whales. The colossal squid, *M. hamiltoni*, was an estimated half to a full trophic level higher than the sperm whales. Furthermore, the $\delta^{13}\text{C}$ values of the majority of cephalopod species supported an overall expected trophic discrimination of $\sim 1-2\text{‰}$ between predator tissue and prey (de Niro & Epstein, 1978). The greater variation within the Bakers Beach stranding may be a result of variation due to individual differences as suggested previously. However, overall with the exception of a

few squid species that had the lowest corrected $\delta^{15}\text{N}$ values, most species fell outside the average 3 - 4 ‰ trophic discrimination values expected between predator and prey (Hobson & Welch, 1992). However, this was less pronounced in the Flinders Island stranding with sperm whales recording the highest $\delta^{15}\text{N}$ values compared to the beaks from their stomachs.

Although recent research on captive fed bottlenose dolphins supports the commonly assumed trophic discrimination of ~ 1 ‰ for ^{13}C between predator and prey, discrimination values of 1.5 - 2 ‰ for ^{15}N were at the lower end of the commonly assumed trophic discrimination values of 2 - 5 ‰ (Browning et al. 2014, Gimenez et al. 2016). Furthermore, current work on long-finned pilot whales also proposed that the trophic discrimination factor for those cetaceans might also be lower than expected (Chapter 3) which may be extrapolated to other cetaceans, including sperm whales. When comparing a common food source of sperm whales worldwide, Marcoux et al. (2007) reported a discrimination factor of one trophic level between $\delta^{15}\text{N}$ values of sperm whale skin and the unadjusted values of histioteuthid beaks. However, as they suggested and as indicated previously (see Chapter 2) the beaks of at least two histioteuthids and several other cephalopods are more impoverished in ^{15}N relative to muscle (by ~ 3.7 ‰) and therefore $\delta^{15}\text{N}$ values need to be corrected accordingly. In contrast, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sperm whales and their squid prey, *Dosidicus gigas*, in the Gulf of California fit well with expected predator-prey discrimination values (Ruiz-Cooley, 2004). Unlike the sperm whale study in the Gulf of California, where the diet is dominated by a single squid species, sperm whales in the present study consumed a greater variety of cephalopod prey with differing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The lack of a clear expected trophic discrimination factor, as found in the present study, does not necessarily preclude some organisms as prey since the diet is more diverse in some regions. Due to differential digestion of prey in the stomachs of sperm whales, other organisms such as fish may also be important prey items as documented in other regions of the world (see Harvey et al. 2014). In Tasmanian waters, based on the current data and that of Evans & Hindell (2004a), a mixed diet of squid (i.e. species with both low and high $\delta^{15}\text{N}$ values) is likely for sperm whales.

Sperm whales have been documented as having a predominance of smaller cephalopods in their stomachs, less than 300 mm dorsal mantle length (DML) for whales stranded in Tasmania (Evans and Hindell, 2004a) and less than 400mm DML for whales stranded in Oregon (Harvey et al. 2014) (see also MacLeod et al 2006b, Santos et al. 1999). It is noteworthy that beaks of *H. atlantica* found in the stomachs of sperm whales in this study were at the smaller end of their bimodal size distribution based on LRL (Xavier et al. 2014). MacLeod et al. (2006b) hypothesized that the consumption of smaller prey by sperm whales may be due to the mode of prey capture. Relative to some other toothed whales, such as the common dolphin, sperm whales have reduced dentition and capture their prey using suction. Since many of their cephalopod prey are neutrally buoyant (Clarke 1980) it is likely that hunting and feeding on these slow-moving groups of squid may increase foraging success and be energetically favorable (MacLeod et al. 2006b). The consumption of higher trophic level squid may be a result of the slow lifestyle of some of these species, such as *M. hamiltoni* and other cranchiids. The irregular predation on large cephalopods such as the colossal squid and the giant squid may be an important energy source as it would potentially reduce the number of prey needed to meet the energetic requirements of the sperm whale (Evans & Hindell 2004a, Harvey et al. 2014). Unlike individual cephalopod species, cephalopod communities are not necessarily size-structured trophically (Cherel & Hobson 2005, Cherel et al. 2009a, Chapter 2). Therefore, it is likely that the lack of trophic size-structure within the cephalopod community, the dominance of smaller cephalopods in the diet and the irregular consumption of higher trophic species has an important impact on the overall trophic position of the sperm whale in the Tasmanian region.

CHAPTER FIVE

STABLE ISOTOPES PROVIDE ANSWERS FOR ELUSIVE PREDATOR-PREY INTERACTIONS – BEAKED WHALES STRANDED IN SUBTROPICAL WATERS

Abstract

Although beaked whales Ziphiidae sp. make up approximately one fourth of all cetacean species there is a paucity of foraging information for these elusive animals. The stable isotope method was used to assess beaked whale foraging dynamics based on skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for five species; *Ziphius cavirostris* (Cuvier's beaked whale), *Tasmacetus shepherdi* (Shepherd's beaked whale), *Mesoplodon grayi* (Gray's beaked whale), *Mesoplodon hectori* Hector's beaked whale, *Mesoplodon layardii* (strap-toothed beaked whale) and one unidentified Ziphiidae sp. that stranded in the coastal waters off Tasmania, Australia between 2001 and 2005. The $\delta^{13}\text{C}$ values for beaked whale skin in this study ranged from -20.4‰ (*C. cavirostris* calf) to -17.6‰ (*T. shepherdi*) reflecting variation in habitat. The $\delta^{15}\text{N}$ values of skin revealed a relatively restricted range (11.0‰ , *C. cavirostris* calf to 13.2‰ , *T. shepherdi*). Based on isotopic values, niche separation was implied with the Gray's and strap-toothed beaked whales foraging in a potentially different habitat than the Hector's and Shepherd's beaked whales. *Teuthowenia pellucida* was numerically the most common cephalopod found in the stomach contents of the Cuvier's beaked whale. Although the corrected cephalopod beak $\delta^{13}\text{C}$ values from the Cuvier's stomach contents confirmed the cephalopods as prey, all but one *Histioteuthis macrohista* beak exceeded the $\delta^{15}\text{N}$ value of the whale (skin 12.1‰). Beak $\delta^{15}\text{N}$ values ranged from 11.4 (*H. macrohista*) to 16.2‰ (*Mesonychoteuthis hamiltoni*). This may suggest that the whale was either supplementing its diet with other prey items or other cephalopod species that were not present in the stomach contents at the time of death. The Tasmanian cephalopod beak $\delta^{13}\text{C}$ values ranged from $-19.3 \pm 0.6\text{‰}$ (*Moroteuthis ingens*) to $-17.2 \pm 0.3\text{‰}$ (*Ancistocheirus lesueuri*) and $\delta^{15}\text{N}$ values ranged from $10.5 \pm 1.1\text{‰}$ (*M. ingens*) to $16.8 \pm 0.7\text{‰}$ (*M. hamiltoni*). Very few of these cephalopod species were potential prey resources for most beaked whales based on isotopic values. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle and liver were analyzed for selected whale species but no clear pattern was documented across species. The lack of a clear pattern highlights the need to use discrimination factors, the relative isotopic difference between a consumer and its prey, that are species and tissue specific (where possible) to be able to provide an accurate description of foraging. Likewise, ontogenetic differences in $\delta^{15}\text{N}$ values of mother-calf pairs also appear to be species-specific with no clear pattern observed in this study. These results provide important information on the diet and pelagic habitat for these seldomly observed beaked whale species.

INTRODUCTION

Beaked whales are considered relatively elusive due to infrequent encounters in the wild (Ferguson et al. 2006), possibly stemming from their deep diving behaviour and accompanying short, surface interval times in oceanic waters (Hilderbrand et al. 2015). However, beaked whales or family Ziphiidae, make up approximately one fourth of all cetaceans (MacLeod et al. 2003). They are often found singly or in small groups of six or less (Hilderbrand et al. 2015). Information relating to the approximate 21 recognized species is scant and primarily gleaned from strandings (Dalebout et al. 2004), although beaked whales only represent a very small percentage of total whale strandings (see Covelo et al. 2016). Nevertheless, commercial hunting for the northern bottlenose whale, *Hyperoodon ampullatus* off the Faroese Islands has resulted in this species being one of the most studied of all beaked whales (Bloch et al. 1996). Cuvier's beaked whale *Ziphius cavirostris* is one of the more common species to strand. It has a worldwide distribution from the ice edge to the equator (MacLeod et al. 2006a). Nevertheless, beaked whales such as the strap-toothed whale *Mesoplodon layardii* and Shepherd's whale *Tasmacetus sherpherdii* likely have a circumpolar distribution in the deep temperate waters of the southern hemisphere (Sekiguchi et al. 1996, MacLeod et al. 2006a, Pitman et al. 2006).

The distribution of a marine predator species within an ecosystem is likely influenced by the distribution of its preferred prey. Furthermore, resource partitioning and competition between species, resulting in subsequent niche separation within an ecosystem, is likely determined by the abundance of prey resources (MacLeod et al. 2003, Spitz et al. 2011). Beaked whales are medium-sized cetaceans, predominantly feeding on oceanic squid and fish with some species having a prevalence of one or the other in their stomachs (e.g. Clarke & Goodall 1994, Santos et al. 2001b, Santos et al. 2007, Spitz et al. 2011, Wenzel et al. 2013). Additionally, oceanic crustaceans have also been recorded in stomachs of *Ziphius cavirostris* (MacLeod et al. 2003). Much of the available foraging information relates to three genera, *Mesoplodon*, *Ziphius* and *Hyperoodon* (e.g. Sekiguchi et al. 1996, Santos et al. 2007, Spitz et al. 2011, Wenzel et al. 2013,

Fernández et al. 2014). There is less dietary information available for the *Tasmacetus*, *Indopacetus* and *Berardius* genera (MacLeod et al. 2003, Best et al. 2014). However, for a single specimen of *T. shepherdi* stranded off Tristan da Cunha *Histioteuthis atlantica*, *Taningia danae*, *Ommastrephes bartrami* and *Pholidoteuthis* 'A' comprised 79 % of the reconstituted mass of cephalopods in the whale stomach (Best et al. 2014). Despite the paucity of information on the trophic dynamics of beaked whales in general, stomach content analysis has provided foundational information for selected species (see MacLeod et al. 2003).

Stomach content analysis is acknowledged as having a number of shortfalls, including the fact that it is a recent 'snapshot' of the predator's diet that may or may not be representative of a whale that has stranded due to unidentified causes (Evans & Hindell 2004a). Moreover, secondary ingestion, i.e. prey of prey, or differential digestion with preferential retention of hard parts such as cephalopod beaks cannot be discounted (Young et al. 2015a). On the other hand, a simultaneous analysis involving stable isotopes and stomach contents of a deepwater oceanic predator and its prey can provide a wider interpretation than is achievable using just one method alone (Hoving et al. 2014).

Stable isotope analysis is an important ecological tool used to help elucidate the trophic ecology, habitat use and migratory patterns of marine species that are logistically challenging to study. Moreover, the ability to differentiate foraging habitats among free ranging species whether over short or long distances is important for their conservation (Hobson 1999). Isotopes can be used to study diet since the isotopic signature of marine species is directly connected to the signature of their prey (de Niro & Epstein 1978, 1981). Typically, $\delta^{13}\text{C}$ values vary at the base of the food chain due to fluctuations in nutrient cycling but are comparatively consistent across trophic levels, thus carrying a signature mirroring isotopically distinct foraging habitats (deNiro & Epstein 1981, Hobson 1999, Cherel & Hobson 2007, Layman et al. 2012). On the other hand, $\delta^{15}\text{N}$ values vary in predictable step-wise enrichment with trophic transfer, approximately 2 - 5 ‰ between each trophic level (Post 2002). Consequently, documenting the relative trophic position between consumers, based on $\delta^{15}\text{N}$

values, provides a means to identify potential niche overlap and competition for resources between the consumers (e.g. Aurióles-Gamboa et al. 2013, Staudinger et al. 2014, Liu et al. 2015).

The expression of dietary prey in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is tissue specific (Borrell et al. 2013b). Stable isotopes of various marine mammal tissues mirror the pooled effects of metabolism (the balance between anabolism and catabolism) and growth rate (time for new tissue accretion) (Fry & Arnold 1982). Higher isotopic turnover is found in more metabolically active tissues (such as liver and blood) and hence echoes the diet of the consumer over a shorter and more recent time period. Conversely, tissues with lower metabolic rates such as muscle and collagen will reflect diet over a longer time period (Tieszen et al. 1983, Hobson & Clark 1992, MacNeil et al. 2005, Malpica-Cruz et al. 2012). The time period denoted by these tissues may represent days to months to years contingent on the renewal rate of the tissue (Borrell et al. 2013b). Therefore, evaluating several tissues of an organism simultaneously may afford foraging information over various time scales (e.g. Kurle & Worthy 2002, MacNeil et al. 2005). Additionally, as a consequence of differing turnover rates, the discrimination factors that are differences in the isotopic value of consumers relative to their prey, are also expected to vary depending on the tissue examined (Caut et al. 2009). This is a critical consideration when comparing the isotopic values of the consumer to the prey (Horstmann-Dehn et al. 2012).

Beaked whales, although few in number are among the various species of cetaceans that strand in Tasmanian waters. The purpose of this study was to use stable isotope analysis to contribute to the understanding of the trophic dynamics of beaked whales particularly in the subtropical waters around Tasmania, off southern Australia. The objectives of the study were 1) to provide an estimate of the trophic position of beaked whales relative to other cetaceans in this region, 2) to determine the prey preference of beaked whales and 3) to determine if isotopic values of tissues with different isotopic turnover rates document dietary shifts over time.

METHODS

Samples of beaked whales for this study were acquired from archived material held by the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE). These samples were obtained from eight individual strandings in Tasmanian waters from 2001 to 2005 using standardized sampling protocol (Geraci & Lounsbury 2005) (Figure 5.1). Dorsal skin and muscle samples were taken from a standardized body site just anterior to the dorsal fin. Whale skin samples of approximately 1 cm² were subsampled from frozen blocks of skin and blubber, held in a -20° C freezer. The 1 cm² samples of skin were then cleaned and rinsed with distilled water after any remaining subcutaneous adipose tissue was removed with a scalpel. Similarly, clean 1 cm³ samples of liver and muscle, when available, were also subsampled from larger frozen blocks and subsequently rinsed in distilled water. All samples were then frozen in preparation for isotopic analysis. Animals were measured for total length (cm) by using a straight line from the tip of the rostrum to the deepest part of the notch in the tail fluke. Furthermore, each individual was sexed and assigned to an age/maturity class (adult, subadult and juvenile/calf) based on their length and maturity.

Samples were also obtained for other potential dietary cephalopod resources of beaked whales. All whole specimens of squid were collected incidentally each fishing season from the deepwater commercial trawl fishing industry operating off southern and eastern Tasmania from 2001 to 2006. The sample of *Nototodarus gouldi* was obtained from a commercial fishermen operating in nearshore Tasmanian waters (see Chapter 2). Upon capture, cephalopods were either immediately frozen on board ship or packed fresh on ice until arrival at the port. Subsequently, the frozen squid were stored in a -20°C freezer until further analysis while the ice-packed fresh squid were immediately frozen or dissected then frozen until analysis (see Chapter 2). Beaks of other cephalopod species were obtained from stomach contents of long-finned pilot whales *Globicephala melas edwardii* (Chapter 3) and sperm whales *Physeter macrocephalus* (Chapter 4) that mass stranded in Tasmanian waters. Specimens of *Slosarczykovia circumantarctica* and *Histioteuthis eltaninae* which were used

as isotopic references for Macquarie Island waters were collected on research cruises around Macquarie Island in June 2000 and December 2000/January 2001 respectively (Chapter 2).

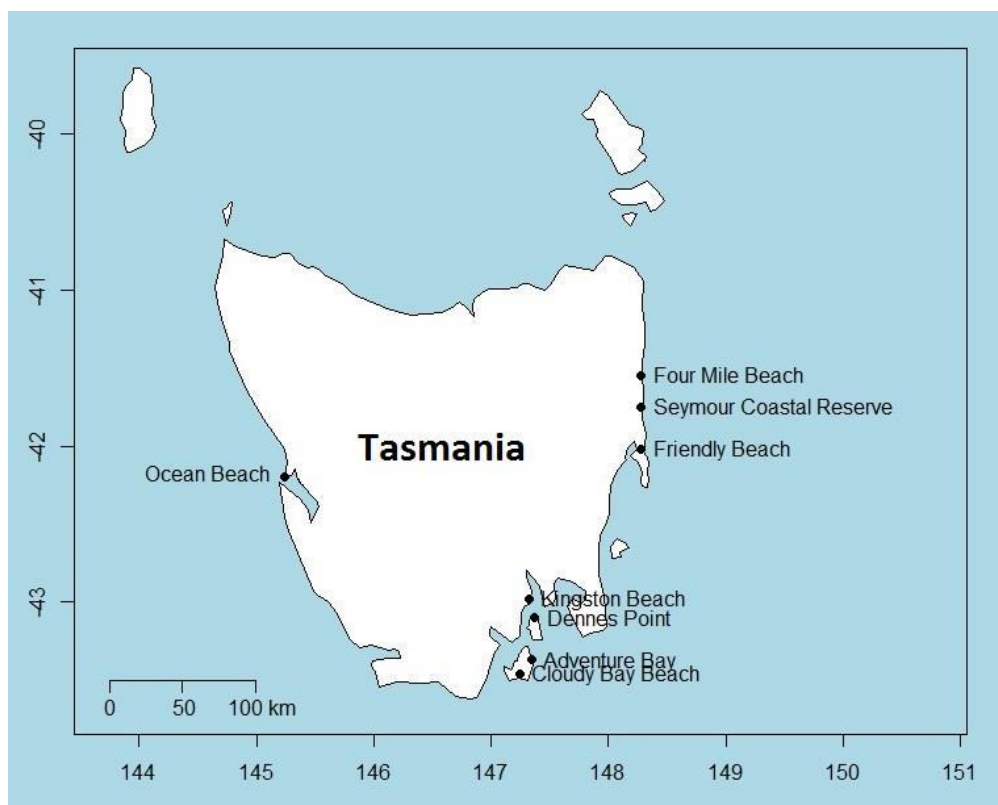


Figure 5.1. Map of beaked whale stranding sites around Tasmania.

When stomach contents for beaked whales were available, cephalopod beaks were separated and archived in 70 % ethanol. Only cephalopod beaks were used in this study and they were identified to species level where possible (Clarke 1986, Xavier & Cherel 2009). Lower rostral lengths (LRL, nearest mm) were determined using digital calipers. Stomach contents that contained cephalopod

beaks were only available for an adult *Ziphius cavirostris* and *Tasmacetus sherpherdii*.

Stable isotope analysis

Just prior to isotopic analysis samples of skin, muscle and liver were freeze dried, ground to a small powder with a Wig-L-Bug® and delipidated in cyclohexane. The ground whale skin, muscle and liver samples along with 3 ml of cyclohexane were left to stand overnight in small covered test tubes under a fume hood. After delipidating overnight, samples were then centrifuged and the supernatant removed with a pipette. Samples were subsequently subjected to two more cyclohexane rinses. Between rinses the samples were allowed to stand for another 1 - 2 hours. Subsequent to delipidation, samples were left to dry uncovered under a fume hood overnight.

All beaks were removed from ethanol and cleaned with distilled water. A small section of the wing tip in the direction of growth was then cut and rinsed with distilled water. The tip of the cephalopod wing represents the most recent growth of the beak and correspondingly the most recent somatic growth of the cephalopod (Cherel & Hobson 2005). Isotopic analysis was undertaken on whole wing tip samples or half wing tip samples for larger specimens.

Except for *Teuthowenia pellucida* where a subsample was taken based of lower rostral length (LRL) size mode, isotopic analysis was conducted on all beaks from stomach contents. The relative abundance of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes was determined by a Finnigan Delta Plus Advantage stable isotope-ratio mass spectrometer at the University of Victoria, British Columbia, Canada. There was a 10 percent replication measurement in each isotopic sample run. The results of the isotopic analysis are presented in the usual delta notation relative to Vienna PD belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 (AIR) for $\delta^{15}\text{N}$. Replicate measurements of internal laboratory standards (DORM) indicated measurement errors of ± 0.1 and ± 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Statistical analysis

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the skin from beaked whales were compared to assess the trophic niche of beaked whale species in the Tasmanian region. All cephalopod beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were corrected to represent muscle since relative to muscle, the tip of the beak wing are approximately 0.7 ‰ more enriched in ^{13}C and 3.8 ‰ more depleted in ^{15}N (Chapter 2). When available $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of whale tissues (muscle, liver, skin) were compared to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of stomach contents (cephalopod beaks) from the same individual to determine if cephalopods found in the whale's stomach are an important part of their diet. All beaked whales were compared with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of potential cephalopod prey previously caught in Tasmanian waters as well as from selected beaks from the stomachs of pilot whales and sperm whales stranded in the same waters (see Chapter 2, 3, & 4). Additionally, the $\delta^{13}\text{C}$ values of *Histioteuthis eltaninae* (-21.8 ± 0.5 ‰) and *Slosarczykovia circumantarctica* (-23.4 ± 2.1 ‰) from Macquarie Island were used as isotopic comparisons for that region. Various tissues from the same individual, with different isotopic turnover rates, were also compared to ascertain if there were shifts in diet over time. All values are means \pm standard deviation, where applicable.

RESULTS

Stable isotopes of ten beaked whales encompassing five different species as well as one unidentified beaked whale species were analyzed (Table 5.1). The lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for beaked whales in this study were for Cuvier's beaked whale *Ziphius cavirostris* (-20.4 and 10.9 ‰, respectively) while Shepherd's beaked whale *Tasmacetus shepherdi* had the highest values (-17.6 and 13.2 ‰, respectively) (Table 5.2). The Shepherd's beaked whale and Hector's beaked whale *Mesoplodon hectori* appeared to have overlapping $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as did the strap-toothed whales *Mesoplodon layardii*, and Gray's beaked whale *Mesoplodon grayi* (Figure 5.2).

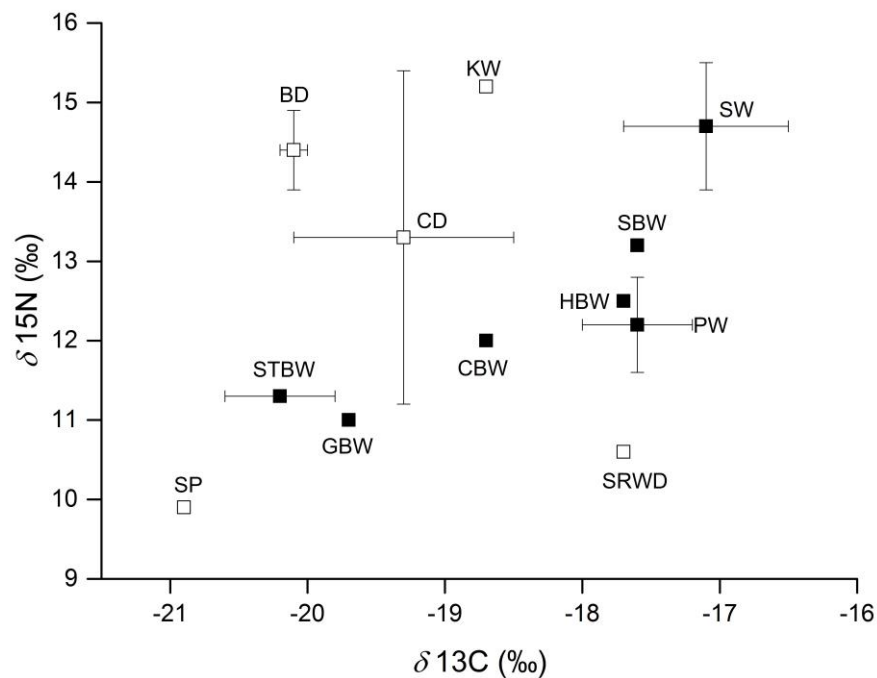


Figure 5.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of skin (filled symbols) and muscle (open symbols) for various cetacean species that stranded in Tasmanian coastal waters. All open squares are from Davenport & Bax, 2002. Long-finned pilot whales and sperm whales are from Chapter 3 & 4. Abbreviations, BD: bottlenose dolphin, CBW: Cuvier's beaked whale, CD: common dolphin, GBW: Gray's beaked whale, HBW: Hector's beaked whale, STBW: strap-toothed beaked whale, KW: killer whale, PW: long-finned pilot whale, SBW: Shepherd's beaked whale, SP: spotted porpoise, SRWD: southern right whale dolphin, SW: sperm whale. Values are means \pm SD.

Table 5.1. Stranding details (Tasmania, Australia) for all beaked whale species in this study, along with sex, maturity and length of specimens for each species. ND = no data.

Species	Common Name	Date of Stranding (d/m/y)	Location	Sex	Maturity	Length (cm)
<i>Ziphius cavirostris</i>	Cuvier's	20/03/05	Ocean Beach	F	adult	578
		20/03/05	Ocean Beach	M	calf	272
<i>Tasmacetus shepherdi</i>	Shepherd's	16/06/03	Friendly Beaches	ND	adult	ND
<i>Mesoplodon grayi</i>	Gray's	27/12/02	Cloudy Bay Beach, Bruny Island	M	adult	512
<i>Mesoplodon hectori</i>	Hector's	13/03/01	Four Mile Beach	F	adult	430
<i>Mesoplodon layardii</i>	Strap-toothed	19/01/04	Bruny Island	F	adult	552
		29/04/04	Seymour Coastal Reserve	F	adult	592
		05/01/05	Bruny Island	F	adult	540
		05/01/05	Bruny Island	M	calf	357
Ziphiidae sp.	Unidentified	18/04/05	Kingston Beach	F	calf	235

Table 5.2. Skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all beaked whales along with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle and liver for selected species.

Species	Maturity	n	skin			muscle			liver		
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio
<i>Ziphius cavirostris</i>	adult	1	-18.7	12.0	3.2	-18.9	12.0	3.1	-20.6	10.7	3.3
	calf	1	-20.4	10.9	3.2						
<i>Tasmacetus shepherdi</i>	adult	1	-17.6	13.2	3.3						
<i>Mesoplodon grayi</i>	adult	1	-19.7	11.0	3.1	-18.2	12.5	3.0	-20.0	10.7	3.4
<i>Mesoplodon hectori</i>	adult	1	-17.7	12.5	3.1						
<i>Mesoplodon layardii</i>	adult	3	-20.1 ± 0.4	11.3 ± 0.4	3.2 ± 0.2				-19.7	10.2	3.1
	calf	1	-20.1	11.7	3.1				-19.7	12.5	3.3
Ziphiidae sp.	calf	1	-18.4	12.4	3.0	-17.0	14.5	3.2	-17.8	13.3	3.2

The $\delta^{13}\text{C}$ values were almost identical for the adult strap-toothed whale between liver and skin. However, skin was more enriched in ^{15}N than liver (+ 0.8 ‰) in the adult female but the reverse was true for the calf (- 0.8 ‰ respectively). Moreover, the calf had an almost identical $\delta^{13}\text{C}$ value to its mother but slightly higher $\delta^{15}\text{N}$ value. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the liver and skin of the Gray's beaked whale were almost identical. However, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the muscle were higher than the liver and skin (+ 1.5, - 1.8 ‰, both isotopes). In the unidentified beaked whale Ziphiidae sp., muscle also had the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values but all tissues tended to differ from each other in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Moreover, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Cuvier's beaked whale exhibited similar muscle and skin values but the liver had higher values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (+ 2.0 and + 1.2 ‰ respectively) (Table 5.2).

The ranges of corrected isotopic values of the cephalopod beaks from the Cuvier's beaked whale were from -19.5 (*Histioteuhis macrohista*) to -18.6 ‰ (*Teuthowenia pellucida*) for $\delta^{13}\text{C}$ and 11.4 (*H. macrohista*) to 16.2 ‰ (*Mesonychoteuthis hamiltoni*) for $\delta^{15}\text{N}$. The $\delta^{13}\text{C}$ values fit well with the adult Cuvier's specimen while the $\delta^{15}\text{N}$ values of all of the prey (except for one *H. macrohista* beak) were higher than even the most ^{15}N enriched tissue, the skin (12.1 ‰) (Figure 5.3). Conversely, one ommastephid beak found in the stomach of the Shepherd's beaked whale not only had a $\delta^{13}\text{C}$ value that fit well as prey of the predator (-17.6 ‰ for whale and -18.4 ‰ for beak) but also had a lower $\delta^{15}\text{N}$ value (11.9 ‰) than the whale predator (13.2 ‰).

Beaked whales were compared with 23 cephalopod species either caught in Tasmanian waters or sampled from the stomach of sperm and long-finned pilot whales that stranded in Tasmanian waters. Species were purposely placed in trophic sequence as opposed to taxonomic sequence, based on increasing corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cephalopod beaks and beaked whales to determine potential prey species of the beaked whale species. Two additional squid comparison species *H. eltaninae* (-21.8 ± 0.5 ‰) and *S. circumantarctica* (-23.4 ± 2.1 ‰) caught around Macquarie Island had the lowest corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The Tasmanian cephalopod corrected $\delta^{13}\text{C}$ values ranged from -19.3 ± 0.6 (*Moroteuthis ingens*) to -17.2 ± 0.3 ‰ (*Ancistrocheirus lesueurii*) and

corrected $\delta^{15}\text{N}$ values ranged from 10.5 ± 1.1 (*M. ingens*) to 16.8 ± 0.7 ‰ (*M. hamiltoni*) (Figure 5.4).

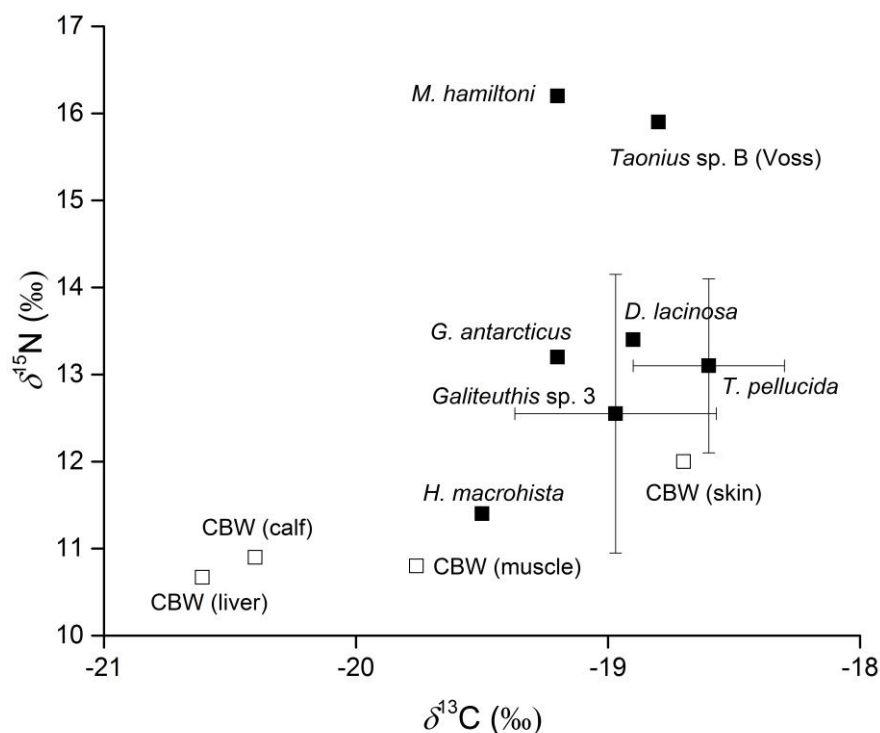


Figure 5.3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the skin, muscle and liver of a female Cuvier's beaked whale (*Ziphius cavirostris*) (open symbols) in comparison to cephalopod beaks (corrected values, see Methods) from its stomach contents (filled symbols). Isotopic values of skin for the female's calf are also given for comparison. *Galiteuthis* sp. 3, *Galiteuthis* sp. 3 (Imber); CBW: Cuvier's beaked whale. Values are means \pm SD.

The strap-toothed whales, Gray's beaked whale and one of the Cuvier's beaked whales had the lowest $\delta^{13}\text{C}$ values, although their values were close to some cephalopod species such as *M. ingens* and *Octopoteuthis* sp. (Figure 5.4). Moreover, only two Tasmanian cephalopod species (*Lycoteuthis lorigera* and *M. ingens*) had corrected $\delta^{15}\text{N}$ values low enough to be important prey items in the diet of strap-toothed whales, Gray's beaked whale and one Cuvier's whale (Figure 5.5).

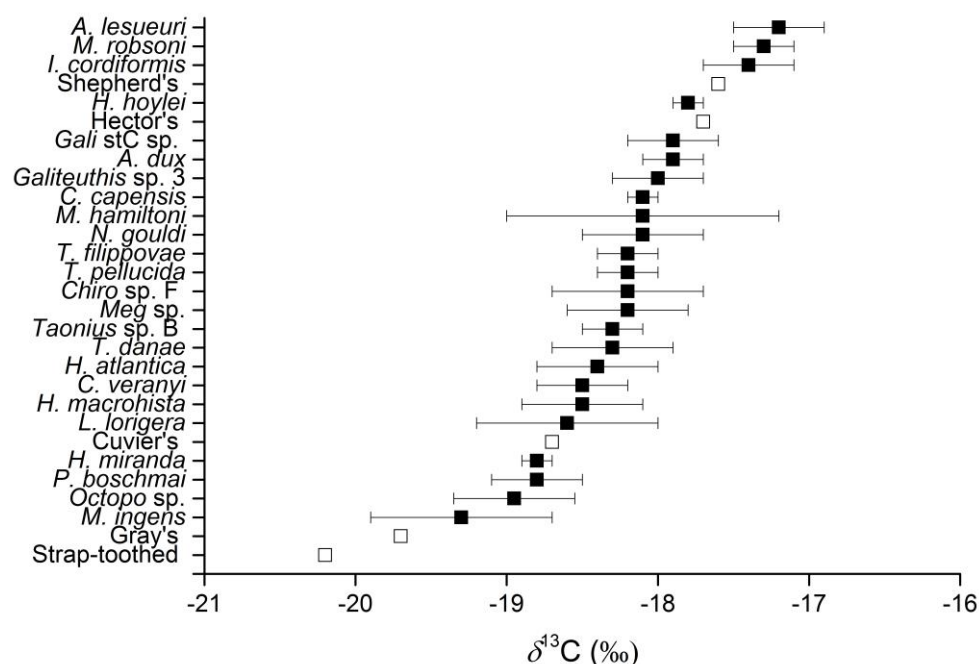


Figure 5.4. $\delta^{13}\text{C}$ values of lower beak wings of cephalopods (corrected values, see Methods) captured in Tasmanian waters as well as from the stomach contents of sperm whales and long-finned pilot whales stranded in coastal waters around Tasmania. Open symbols are skin $\delta^{13}\text{C}$ values from beaked whale species. Abbreviations, *Octopo* sp.: *Octopoteuthis* sp., *Taonius* sp. B: *Taonius* sp. B (Voss), *Meg* sp.: *Megalochranchia* sp., *Chiro* sp. F: *Chiroteuthis* sp. F (Imber), *Galiteuthis* sp. 3: *Galiteuthis* sp. 3 (Imber), *Gali* stC sp.: *Galiteuthis* stC sp. (Imber). Values are means \pm SD.

The Hector's beaked whale and Shepherd's beaked whale shared some of the highest values of $\delta^{13}\text{C}$ with other cephalopods such as *Histioteuthis hoylei* and *Galiteuthis* stC sp. (Imber). Therefore, many cephalopod species would fit as possible prey based on corrected $\delta^{13}\text{C}$ values (Figure 5.4). However, although Hector's and Shepherd's beaked whale shared similar $\delta^{15}\text{N}$ values with some cephalopod species such as *Octopoteuthis* sp. and *P. boschmai* only a few species would be potential important prey items based on corrected $\delta^{15}\text{N}$ values (e.g. *Todarodes filippovae*, *H. macrohista*, *L. lorigera* and *M. ingens*) (Figure 5.5).

DISCUSSION

Beaked whale species in this study showed considerable variation in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Nevertheless, there was a large amount of overlap in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of some species with values obtained for several odontocetes in a previous study from this region (Davenport & Bax 2002). This infers a potential trophic overlap between some predators within the pelagic habitat. Based on

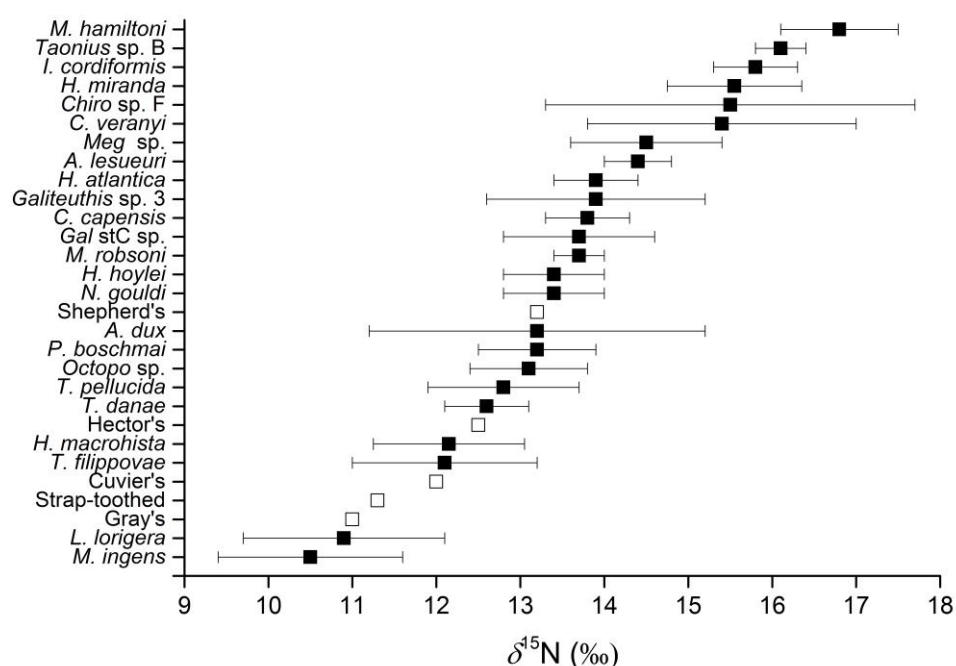


Figure 5.5. $\delta^{15}\text{N}$ values of lower beak wings of cephalopods (corrected values, see Methods) captured in Tasmanian waters as well as from the stomach contents of sperm whales and long-finned pilot whales stranded in coastal waters around Tasmania. Open symbols are skin $\delta^{15}\text{N}$ values from beaked whale species. Abbreviations, *Octopo* sp.: *Octopoteuthis* sp., *Gali stC* sp.: *Galiteuthis* stC sp. (Imber), *Galiteuthis* sp. 3: *Galiteuthis* sp. 3 (Imber), *Meg* sp.: *Megalochranchia* sp., *Chiro* sp. F: *Chiroteuthis* sp. F (Imber), *Taonius* sp. B: *Taonius* sp. B (Voss). Values are means \pm SD.

$\delta^{15}\text{N}$ values of beaked whales were an estimated one to two trophic levels lower relative to some predators that had similar $\delta^{13}\text{C}$ values. For example, strap-toothed whales and the Gray's beaked whale were lower than bottlenose

Tursiops truncatus dolphins, the Cuvier's beaked whale was lower than the killer whale *Orcinus orca* (Davenport & Bax 2002) and the Shepherd's beaked whale and Hector's beaked whale, which were similar to pilot whales *Globecephala melas edwardii*, were lower than the sperm whales *Physeter macrocephalus* (Chapter 3, 4, Figure 5.5). Likewise, Auriolles-Gamboa et al. (2013) documented that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beaked whales in the Gulf of California overlapped with other odontocetes such as sperm and pilot whales, as well as bottlenose dolphins. Nevertheless, the beaked whales in this study appeared to segregate into two main groupings conceivably representing separate niches. Strap-toothed and Gray's beaked whales were more depleted in ^{13}C and ^{15}N than the Shepherd's and Hector's beaked whales which both shared comparable values to long-finned pilot whales that stranded in the same waters (see Chapter 3). The adult Cuvier's whale had intermediate values to these whales.

MacLeod et al. (2003) in their review of beaked whale prey consumption, including prey size based on stomach content examination, proposed that *Mesoplodon* sp. and *Ziphius* sp. appear to inhabit separate niches. They documented that *Mesoplodon* sp. had the highest representation of fish in their stomachs, with some species' stomachs comprised exclusively of fish. In contrast, Cuvier's beaked whale stomachs virtually always comprised squid, and rarely if ever, contained fish (also see Santos et al. 2007). Furthermore, the squid prey in *Mesoplodon* sp. were smaller (< 500 g) compared to the Cuvier's beaked whale (> 1000g) (MacLeod et al. 2003). In contrast, prior studies on Gray's beaked whale have not noted any cephalopods in their diet (Sekaguchi 1994 cited in MacLeod et al. 2003). In strap-toothed whales from South Africa and New Zealand waters over 94% of recorded items in stomach contents were cephalopods, with the predominant species being *Histoteuthis* sp. and *Taonius pavo* along with unidentified fish and crustaceans (Sekaguchi et al. 1996). Although no stomach contents were available for the strap-toothed and Gray's beaked whales in the present study, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values indicate an overlap in their foraging habitats, but likely in different habitats than sperm whales and long-finned pilot whales. Furthermore, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the cephalopods from the Tasmanian region do not fit well as potential prey for these two beaked whale species.

Strap-toothed and Gray's beaked whales are likely distributed in temperate waters as far south as 63 °S, and further south to Antarctic waters, respectively (MacLeod et al. 2006a).

On the other hand, the more enriched isotopic values of the Hector's and Shepherd's beaked whales infer an analogous foraging habitat to that of long-finned pilot whales from the Tasmanian region. Long-finned pilot whales are believed to have a diet consuming both cephalopods and fish probably in oceanic waters and along the continental slope (see Chapter 3). Although the diet of Hector's beaked whales is relatively unknown, a recent dietary evaluation of a solitary specimen of a Shepherd's beaked whale stranded off Tristan da Cunha in the South Atlantic acknowledged the presence of both fresh fish remains as well as cephalopod beaks (including *Histioteuthis atlantica*, *Taningia danae*, *Ommastrephes bartrami* and *Pholidoteuthis* 'A') (Best et al. 2014). The authors hypothesized that this species may alternately forage on fish and squid contingent on access to seamounts or the continental slope. The corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the ommastrephid *T. filippovae* caught in slope waters off Tasmania fit well as a potential food resource for the Shepherd's beaked whale stranded in Tasmanian waters. Moreover, a single Ommastrephidae sp. beak retrieved from the stomach of the Shepherd's beaked whale also suggests that this whale may have potentially foraged over the continental slope. More data is needed to confirm this theory.

The widespread geographical distribution of Cuvier's beaked whale in warm-temperate to tropical waters as well as the higher number of strandings reported for this species has in part afforded a relatively substantial dietary data set (MacLeod et al. 2003, Santos et al. 2007). Cuvier's beaked whale is primarily assumed to be a teuthophagous predator specializing on mesopelagic and bathypelagic species consistent with its oceanic habitat (e.g. Santos et al. 2007). Both numerically and in terms of biomass, Histioteuthidae, Cranchiidae and Gonatidae are the most important cephalopod families found in stomachs of Cuvier's beaked whales (MacLeod et al. 2003). In the specimen examined in this study, the deep sea Cranchiidae, *Teuthowenia pellucida* was the most important numerically. *T. pellucida* has been caught in Tasmanian waters (Chapter 2) and

correspondingly *Teuthowenia* sp. was deemed an important prey item for a Cuvier's beaked whale investigated from New Zealand waters (Fordyce et al. 1979). In Scottish waters Cuvier's beaked whales have been compared to sperm whales in terms of scope of prey consumed (Santos et al. 2001b). Sperm whales in the Tasmanian region also ingest similar cephalopod species (Chapter 4) to that consumed by the beaked whale in this study. However, even though sperm whales had higher $\delta^{15}\text{N}$ values than the Cuvier's beaked whale, their differing $\delta^{13}\text{C}$ values preclude a direct trophic comparison (see Figure 5.5). Despite similar $\delta^{13}\text{C}$ values to that of its prey the corrected $\delta^{15}\text{N}$ values of all the cephalopod prey (except for one *H. macrohista*) from the stomach of the Cuvier's whale in this study (11.5 to 16.2 ‰) exceeded all $\delta^{15}\text{N}$ values from any of the whale's tissues (i.e. liver, muscle or skin, 10.7 to 12.0 ‰). Moreover, this beaked whale consumed two species of cephalopods (*Taonius* sp. B (Voss) and *M. hamiltoni*) that have two of the highest $\delta^{15}\text{N}$ values for this region (Chapter 4). *Taonius* sp. B (Voss) and *M. hamiltoni* are cranchiids which are probably slow moving (Rosa & Seibel 2010, Burford et al. 2015) and hence easy prey for a whale that forages by suctioning-feeding (MacLeod et al. 2006b). *M. hamiltoni* was also noted in the stomach of a Cuvier's beaked whale stranded off New Zealand (Fordyce et al. 1979). However, since none of the cephalopod species found in the stomach of the Cuvier's beaked whale in this study fit the expected trophic discrimination between predator and prey based on ^{15}N (2- 5 ‰) (Post 2002) it is likely that additional lower trophic organisms (including other cephalopods) may be key prey items for Cuvier's beaked whale in this region. Crustaceans may be an important prey item for this whale species (MacLeod et al. 2003). Alternatively, the beaks in the stomachs may not be representative of its normal foraging habitat, although similar $\delta^{13}\text{C}$ values would suggest otherwise.

A small fishery targeting Cuvier's beaked whales in Japanese waters afforded an opportunity to extract dietary information related to water depth (Nishiwaki & Oguro 1972). It was noted that this species of beaked whale principally consumed cephalopods at depths less than 1000 m but switched to fish at depths greater than 1000 m. Nishiwaki and Oguro (1972) postulated that Cuvier's beaked whales are opportunistic foragers. The range of prey species found in the

present study as well as other studies (see Blanco & Raga 2000, MacLeod et al. 2003, Santos & Pierce, 2005, Santos et al. 2007, Spitz et al. 2011) along with $\delta^{15}\text{N}$ values of cephalopod prey that exceed that of the predator implies that the whale is likely a generalist feeder targeting abundant species within its foraging path (MacLeod et al. 2003).

From a methodological point of view, it is crucial when considering potential food sources for consumers to use discrimination factors that are both tissue and species specific where possible. This is because the isotopic difference between the consumer and its prey may vary according to the species or tissue being examined (Caut et al. 2009). Skin is a common tissue used in cetacean research principally due to non-lethal methods of attaining samples such as biopsy darts and collection of skin sloughs (e.g. Ruiz-Cooley et al. 2004, Marcoux et al. 2007, Ryan et al. 2012, Hunt et al. 2013). However, muscle tissue is still one of the tissues of choice for isotope studies despite its slower turnover rate than plasma for example, which is likely to reflect recent feeding (Tieszen et al. 1983, Vander Zanden et al. 2015). Horstmann-Dehn et al. (2012) reported that skin was generally more enriched in ^{15}N than muscle for bowheaded whales *Balaena mysticetus* and gray whales *Eschrichtius robustus* but not for belugas *Delphinapterus leucas*. Likewise, the skin of long-finned pilot whales has also been documented to be more enriched in ^{15}N than muscle (Abend & Smith 1997, Fontaine et al. 2015). Nevertheless, Borrell et al. (2013b) found that the discrimination factors for ^{15}N between skin and muscle of fin whales *Balaenoptera physalus* and their prey (euphasiid krill, *Meganyctiphanes norregica*) was similar. These contrasting results highlight the need to validate the discrimination factors between tissues of consumers and their prey for the species being studied where possible (Caut et al. 2009).

Analysis of multiple tissues from the same animal simultaneously may provide a way to understand cetacean feeding on a larger temporal scale than the examination of a single tissue (Hobson et al. 1996). Recent experiments on bottlenose dolphins held in captivity on a constant diet have shown that their skin had a half life of 48 days for nitrogen, representing a turnover rate of up to six months (95%) (Giménez et al. 2016). This is in contrast to internal organs

such as liver that have a much shorter turnover rate and consequently represent foraging over a shorter time period (Boecklen et al. 2011, Vander Zanden et al. 2015). No clear overall pattern was found for the beaked whales in this study except that different tissues likely represent changes in foraging. The strap-toothed whale that had similar $\delta^{13}\text{C}$ values for the liver and skin was possibly consistently foraging in a similar habitat over an extended period of months. In contrast, the metabolically active liver that is likely to have a shorter turnover than skin (Tieszen et al. 1983, Martínez del Rio & Carleton 2012) and was more depleted in ^{15}N might imply a shift in prey. Similarly, the Cuvier's beaked whale had similar isotopic values between muscle and skin but the liver was more depleted in ^{13}C and ^{15}N suggesting a recent change in foraging habitat. Furthermore, the unidentified Ziphiidae sp. may be a more mobile species since all three tissues were different, with muscle being the most enriched in both ^{13}C and ^{15}N .

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mother-calf pairs demonstrated mixed results. The strap-toothed calf had identical $\delta^{13}\text{C}$ values to the mother but had higher $\delta^{15}\text{N}$ values relative to the mother. Conversely, the Cuvier's calf had lower values in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than its mother. While size or maturity is often associated with increasing $\delta^{15}\text{N}$ values (e.g. Lesage et al. 2001, Mendes et al. 2007b) some studies have indicated a decreasing trend in $\delta^{15}\text{N}$ values with size (e.g. sperm whales, Borrell et al. 2013a). However, since mothers catabolize their tissues to produce milk it is generally accepted that calves should display trophic enrichment in ^{15}N relative to the mother (Newsome et al. 2010). Yet the data is mixed with no difference in $\delta^{15}\text{N}$ values between juveniles/calves and their mothers indicated in some studies (e.g. long-finned pilot whales, Chapter 3). However, Cherel et al. (2015) proposed that this trophic enrichment of ^{15}N is likely more visible when $\delta^{15}\text{N}$ values of the tissue from the calf is compared to the mother's milk rather than tissue from the mother.

In conclusion, even though the sample size of the beaked whales in this study is small, this research contributes new information relating to the trophic ecology of these elusive and poorly studied whales. The data supports the hypothesis by MacLeod et al. (2003) that niche separation is likely for some beaked whale

species. The combination of stable isotopes along with stomach content analysis highlighted that some species thought to be teuthophagous (i.e. Cuvier's beaked whale) might also be consuming other organisms. Alternatively they may be feeding on other cephalopods not present in the stomachs of these stranded individuals that impact their isotopic signature and subsequent trophic position. Differential digestion and preferential retention of certain hard parts such as cephalopod beaks may skew the stomach content data towards cephalopods (Santos et al. 2001a, Young et al. 2015a). Furthermore, some beaked whale species may not spend all their time foraging in waters surrounding Tasmania. Continued research is needed to determine if the results here can be generalized within and between species.

CHAPTER SIX

GENERAL DISCUSSION AND FUTURE DIRECTIONS

Methodology - Using predators as biological samplers

This study has highlighted the use of two different techniques used in concert to greatly improve our understanding of the trophic dynamics for cetacean-cephalopod interactions in subtropical waters around Tasmania. Stable isotope analysis is based on the premise that 'you are what you eat' rather than a snapshot of ingested prey as in stomach content analysis. Using predators as biological samplers along with stable isotope analysis can provide information from basic foraging behaviour of a predator to complex community dynamics of their prey over differing temporal and spatial scales. This would normally require significant sampling effort (if even possible) to achieve a similar result if undertaken using conventional methods (Crawford et al. 2008). This is particularly true for the sampling of oceanic cephalopods that have no commercial value, such as large gelatinous or ammoniacal squids. Due to their remote habitat, oceanic cephalopods are less frequently sampled (Staudinger et al. 2013, Xavier et al. 2015). However, oceanic cephalopods in all water masses from the tropics to the poles are important prey resources for many bird, fish and marine mammals (Xavier et al. 2002, Evans & Hindell 2004a, Daneri et al. 2012, Staudinger et al. 2013, Xavier et al. 2014, Guerreiro et al. 2015, Negri et al. 2016, Seco et al. 2016). Teuthophagous predators that ingest a diverse array of cephalopods differing in size and maturity subsequently retain and often accumulate the cephalopod beaks from their prey in their stomachs. The use of opportunistic stranding events of whales for example, can provide access to cephalopod beaks that would otherwise be not available. An analysis of the stomach contents of the predator is likely to provide an enhanced picture of the cephalopod community than may be obtained through conventional sampling methods (Staudinger et al. 2013).

The use of predators as biological samplers in this, and other studies, is nevertheless constrained by the predator's trophic interactions including their foraging habit and

prey preference. Many toothed whales are opportunistic teuthophageous predators thus providing a good snapshot of the cephalopod community. However, these snapshots are dependent on the foraging behaviour of the whale species and may reflect seasonal, sex-related or morphological differences. Most strandings of toothed whales in Tasmanian waters occur in the late spring or summer months. Male bull sperm whales *Physeter macrocephalus* only forage in subtropical waters during mating time, normally residing in cooler waters toward the poles (Gosho et al. 1984). Moreover, some toothed whales, such as sperm whales and pilot whales *Globicephala melas edwardii*, are also sexually dimorphic potentially diving and feeding at different depths. Prey size preference as well as beak accumulation and digestion rates may also influence sampling as toothed whale prey selection has been attributed to morphological differences in mode of prey capture (MacLeod et al. 2006b). Smaller beaks from stomach contents may be more difficult to identify due to immature morphological differences compared to mature specimens. Furthermore, smaller squid and octopods are digested more quickly and therefore the beaks are more likely to be damaged in predator stomachs, resulting in them being difficult to identify or excreted by the predator (Staudinger et al. 2013).

Despite the caveats of using predators as biological samplers the use of teuthophageous predators has also provided previously unreported ecological information not only of the prey but also of the consumer themselves. Predator-prey interactions have been documented on the distribution, abundance, trophic level and trophic interactions for a number of teuthophageous predators in different water masses (Cherel et al. 2009a, Roberts et al. 2011, Staudinger et al. 2014, Walters et al. 2014, Seco et al. 2016). Furthermore, Xavier et al. (2013) showed how stomach contents of the grey-headed albatross *Thalassarche chrysostoma* reflected changes in environmental conditions with a shift away from cephalopods to Antarctic krill *Euphausia superba* around South Georgia during the austral summer of 1999/2000 when there were anomalously high sea surface temperatures relative to other years. This clearly demonstrates the usefulness of using cephalopod predators to monitor community changes by assessing the presence and relative

abundance of cephalopods to other prey. Stomach content analysis may also document latitudinal changes in prey distribution that reflects environmental changes due to temperature or other anthropogenic stressors such as overfishing (Staudinger et al. 2013).

The simultaneous use of techniques, such as stomach contents, stable isotopes, fatty acid, heavy metal or molecular genetic analyses can provide powerful results in elucidating the community structure of prey (such as cephalopods) as well as determining the foraging preferences of a predator (Xavier & Croxall 2007, Cherel et al. 2009a, Hoving et al. 2015, Young et al. 2015a). Additional information can be attained that would not otherwise be available if only one technique was applied in the study. For example, due to differential digestion of prey, the presence of only hard parts such as cephalopod beaks in the stomachs of a predator (which may have accumulated over time) could initially indicate that a predator is teuthophageous. However, the isotopic analysis of both the predator and the stomach contents may provide additional information that indicates that the predator's diet is not dominated by cephalopods but supplemented or even dominated by other organisms. The examination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for female southern elephant seals from Kerguelen Islands in conjunction with their main prey revealed they are predominantly myctophid feeders rather than squid feeders as previously thought (Cherel et al. 2008).

Pelagic predators in Tasmanian waters

In marine ecosystems that are characterized by a diversity of odontocetes there is often evidence of considerable overlap as well as segregation between the foraging niches as reflected in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (e.g. Spitz et al. 2011, Aurióles-Gamboa et al. 2013). The foraging niche of odontocetes may be evaluated based on three major dimensions including 1) the trophic level whereby various characteristics of their main prey are examined 2) a spatial level related to their foraging activity and 3) a temporal level related to their movement and diel activity patterns (Spitz et al. 2011).

There was considerable overlap in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the toothed whales in this study with other pelagic marine mammals from the region (Davenport & Bax 2002). A similar overlap in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was also found in the Gulf of California where, not surprisingly, the killer whale *Orcinus orca* appears to be a top predator (Aurioles-Gamboa et al. 2013) similar to that found for the Tasmanian region (based on $\delta^{15}\text{N}$ values from Davenport & Bax 2002). Furthermore, sperm, pilot and beaked whales from the Gulf of California, also exhibited close $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values likely representing niche overlap (Aurioles-Gamboa et al. 2013). In the northwest Mediterranean the difference in ^{15}N enrichment of sperm whales compared to pilot whales was much lower (1 ‰) (Praca et al. 2011) than that found in the present study. Sperm whales stranded around Tasmania were equivalent to approximately one trophic level higher than pilot whales based on $\delta^{15}\text{N}$ values. The high $\delta^{15}\text{N}$ values of sperm whales provide evidence of their higher order predation in the Tasmanian region. The sperm whales also had higher $\delta^{15}\text{N}$ values than Cuvier's (adult) *Ziphius cavirostris*, Shepherd's *Tasmacetus shepherdi*, Ziphiidae sp. and the Hector's beaked whales *Mesoplodon hectori*. However, the Gray's *Mesoplodon grayi* and strap-toothed beaked whales *Mesoplodon layardii* had lower $\delta^{15}\text{N}$ values than the sperm whales but also appeared to be from a different habitat (based on $\delta^{13}\text{C}$ values) and therefore are not comparable (Figure 6.1).

A review of the diet in beaked whales reveals that some species likely have very different dietary niches (MacLeod et al. 2003). The stomachs of the genus *Mesoplodon* (including Gray's, Hector's and strap-toothed beaked whales) generally had smaller cephalopod prey (less than 500 g) compared to that of *Ziphius* (Cuvier's beaked whale). The Cuvier's beaked whale had species with a mean weight of over 1000 g in their stomach contents (MacLeod et al. 2003). Sperm whales have also been documented as having a predominance of relatively smaller cephalopods in their stomachs (Evans & Hindell 2004a, Harvey et al. 2014). However, this present study documented that all whale species analysed had ingested some cephalopods with higher $\delta^{15}\text{N}$ values than themselves, such as *Mesonychoteuthis hamiltoni* and *Taonius* sp. B (Voss).

The irregular predation on larger cephalopods by some whales may reduce the amount of prey needed to meet their energetic requirements at any one time (Evans & Hindell 2004a, Harvey et al. 2014). Although some whales may eat larger cephalopods this does not necessarily assume a higher $\delta^{15}\text{N}$ value or trophic level for the whale since cephalopod communities are not size structured according to trophic level (Cherel & Hobson 2005, Cherel et al. 2009a). For example, two large cephalopod species such as *Taningia danae* and *Lepidoteuthis grimaldi* can have high and low $\delta^{15}\text{N}$ values, respectively (Cherel et al. 2009a).

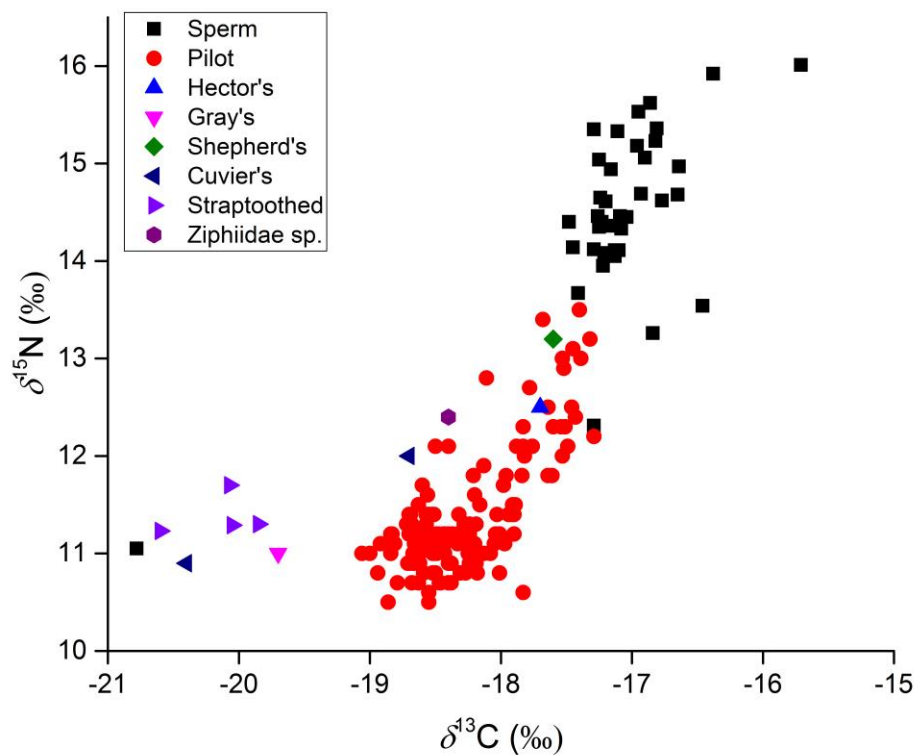


Figure 6.1. Skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all toothed whales from this study stranded in Tasmanian waters

One of the most surprising factors in this study overall was the similar $\delta^{13}\text{C}$ values but clear *lack* of trophic enrichment of $\delta^{15}\text{N}$ of whales over the cephalopod beaks from their respective stomachs. Many cephalopod species had higher $\delta^{15}\text{N}$ values than their whale consumers. Most of the odontocetes in this study, with the

exception of Sherpherd's beaked whale where the diet is largely unknown, are considered to be teuthophagous to a greater or lesser extent (Clarke 1996, MacLeod et al. 2003, Fernández et al. 2009). A plausible explanation for the lack of trophic enrichment is they are also supplementing their diet with lower trophic organisms that are not represented in the stomach contents. This may mean that smaller cephalopods, where the beaks are not retained in the stomach due to being more easily damaged and excreted, or other organisms such as fish, crustaceans or salps are being consumed. This explanation may be particularly relevant to the pilot whales, which have been documented in the northeast Atlantic to also consume fish and salps, sometimes in significant proportions (Spitz et al. 2011). Crustaceans have also been found in Cuvier's beaked whale stomachs in substantial amounts (MacLeod et al. 2003). Cuvier's beaked whales have also been documented as consuming cephalopods at depths less than 1000 m but switching to foraging on fish at depths greater than 1000 m (Nishiwaki & Oguro 1972). On the other hand, sperm whales appear to be almost exclusively teuthophagous with other organisms infrequently found in the stomach contents of hunted or stranded animals (Clarke 1980, Evans & Hindell 2004a). The $\delta^{15}\text{N}$ values of the sperm whales when compared to the stomach contents suggest that their $\delta^{15}\text{N}$ value is dependent on a mixture of lower and upper trophic level cephalopods rather than supplementing with other lower trophic organisms.

Neither stomach content analysis or stable isotopes are able to identify all dietary contributions as there can be multiple combinations of sources all contributing to the observed isotopic value of the consumer (Layman et al. 2012). However, an examination of the isotopic value of the consumer and the prey provides pertinent information on the completeness of the stomach content analysis.

An alternative explanation to why the cephalopod prey of some whale species have $\delta^{15}\text{N}$ values exceeding that of their predator, is that the trophic discrimination factor (the difference between the consumer and its food) may be lower for some whale species than commonly thought. Captive experiments on bottlenose dolphins revealed a trophic discrimination factor closer to 1.5 - 2 ‰ for ^{15}N instead of the 2 -

5 ‰ normally reported (Post 2002, Browning et al. 2014, Giménez et al. 2016).

There was substantial similarity in the cephalopod species represented in the stomach contents of the various toothed whale species examined in this study (Figure 6.2). All toothed whale species consumed a variety of cephalopod species representing a range in $\delta^{15}\text{N}$ values (Figure 6.3). Sperm whales consumed all the same cephalopod species represented in the pilot whale stomachs with the exception of two species (*Lycoteuthis lorigera* and *Chiroteuthis capensis*) (Table 6.1). *L. lorigera* was well represented numerically in the diet of Bicheno and Marion Bay pilot whales. It is also the lowest trophic level cephalopod consumed by any of the toothed whales in this study, which may be partially responsible for the overall lower $\delta^{15}\text{N}$ for pilot whales relative to other whales. However, sperm whales had a much greater diversity of species in their stomachs, with an additional 13 species not consumed by pilot whales. Stranded sperm whales in Tasmanian waters have previously recorded close to 50 cephalopod species (Evans & Hindell 2004a). With the exception of *Gonatus antarcticus* and *Discoteuthis laciniosa* sperm whales also ingested similar cephalopod species to the Cuvier's beaked whale (Table 6.1).

The presence of similar species represented in the stomachs of sperm and Cuvier's whales may be explained by foraging in similar deepwater and bathypelagic habitats. Sperm whales and Cuvier's whales are known to dive to great depths of approximately 1860 m (Watwood et al. 2006, Davis et al. 2007, Teloni et al. 2008) to 3000 m (Schorr et al. 2014) respectively, to forage for prey compared to pilot whales which dive to slightly shallower depths of 360 m (Baird et al. 2002) to over 800 m (Heide-Jørgensen et al. 2002).

Our results concur with previous results for sperm whale diets, with no neritic or inshore cephalopod species found in their stomachs (Fernández et al. 2009). Moreover, the isotopic values of the cephalopod species are more representative of oceanic or bathypelagic species (Das et al. 2003) (Figure 6.2 and 6.4). Similarly, the overall habitat of the cephalopod species from the Cuvier's beaked whale is predominantly oceanic (Figure 6.4).

Table 6.1. Cephalopod prey species found in stomachs of toothed whales stranded in Tasmanian waters. LFPW, Long finned pilot whale *Globicephala melas edwardii*; SW, Sperm whale *Physeter macrocephalus*; CBW, Cuvier's beaked whale *Ziphius cavirostris*; SBW, Shepherd's beaked whale *Tasmacetus shepherdi*.

Family	Species	LFPW	SW	CBW	SBW
Lycoteuthidae	<i>Lycoteuthis lorigera</i>	●			
Enoploteuthidae	<i>Ancistocheirus lesueuri</i>	●	●		
Octopoteuthidae	<i>Octopoteuthis</i> sp.	●	●		
	<i>Taningia danae</i>		●		
Onychoteuthidae	<i>Moroteuthis robsoni</i>		●		
Cycloteuthidae	<i>Cycloteuthis akimushkini</i>		●		
	<i>Discoteuthis laciniosa</i>			●	
Gonatidae	<i>Gonatus antarcticus</i>			●	
Lepidoteuthidae	<i>Lepidoteuthis grimaldi</i>		●		
	<i>Pholidoteuthis boschmai</i>	●	●		
Architeuthidae	<i>Architeuthis dux</i>	●	●		
Histiotteuthidae	<i>Histiotteuthis atlantica</i>	●	●		
	<i>Histiotteuthis hoylei</i>		●		
	<i>Histiotteuthis macrohista</i>			●	
	<i>Histiotteuthis miranda</i>		●		
Ommastrephidae	Ommastrephidae sp.	●	●		●
Chiroteuthidae	<i>Chiroteuthis</i> sp. F (Imber)		●		
	<i>Chiroteuthis capensis</i>	●			
	<i>Chiroteuthis veranyi</i>		●		
Cranchiidae	<i>Taonius</i> sp. B (Voss)		●	●	
	<i>Teuthowenia pellucida</i>	●	●	●	
	<i>Megalocranchia</i> sp.	●	●		
	<i>Galiteuthis</i> sp. 3 (Imber)		●	●	
	<i>Galiteuthis</i> stC sp. (Imber)		●		
	<i>Mesonychoteuthis hamiltoni</i>		●	●	

In contrast, pilot whales have often been recorded as foraging in both shelf and oceanic habitats (Fernández et al. 2009). Some stranded individuals have had neritic species represented in their stomach contents (Gales & Pemberton 1992, Beatson et al. 2007a). In the Bay of Biscay in the northeast Atlantic, pilot whales were reported

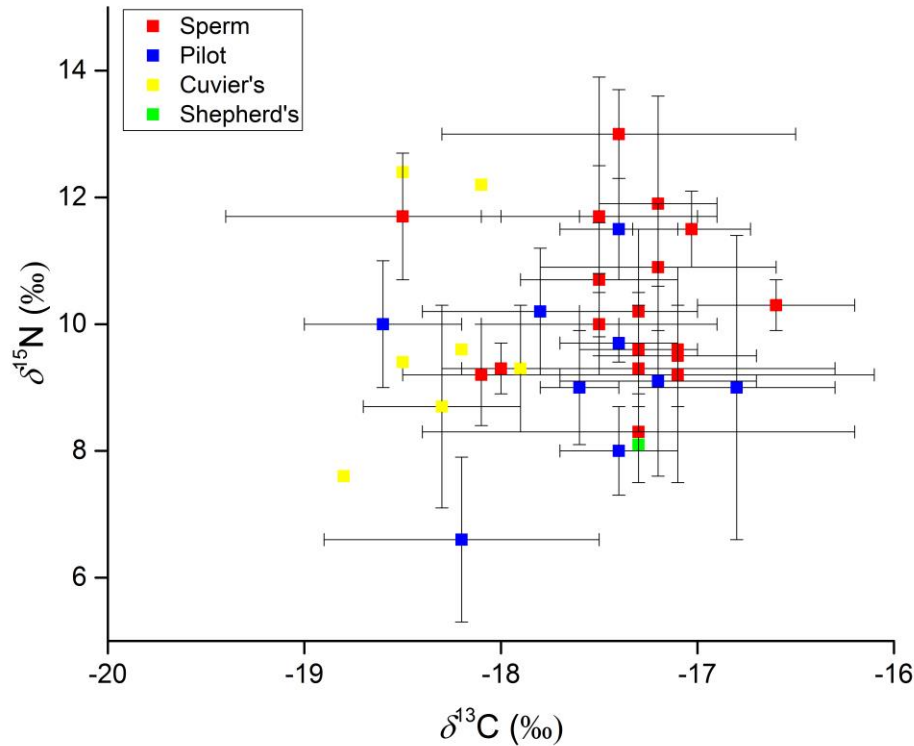


Figure 6.2. Mean \pm SD of cephalopod beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all species consumed by Sperm *Physeter macrocephalus*, Pilot, *Globicephala melas edwardii* Cuvier's *Ziphius cavirostris* and Shepherd's beaked whales *Tasmacetus shepherdi* in this study.

to have ingested both oceanic and neritic cephalopod species (Spitz et al. 2011). However, it is always uncertain as to whether stranded individuals have fed normally in the days prior to their death. Comparison of isotopic values of the whales to their stomach contents can help illuminate this uncertainty. There was an absence of any neritic cephalopod species in the stomachs of the stranded pilot

species in this study. Furthermore, the isotopic value of the cephalopods and the whales indicated a slope or oceanic habitat. Nevertheless, it is possible some pilot whales may have been feeding over the outer shelf. Overall, although there was an

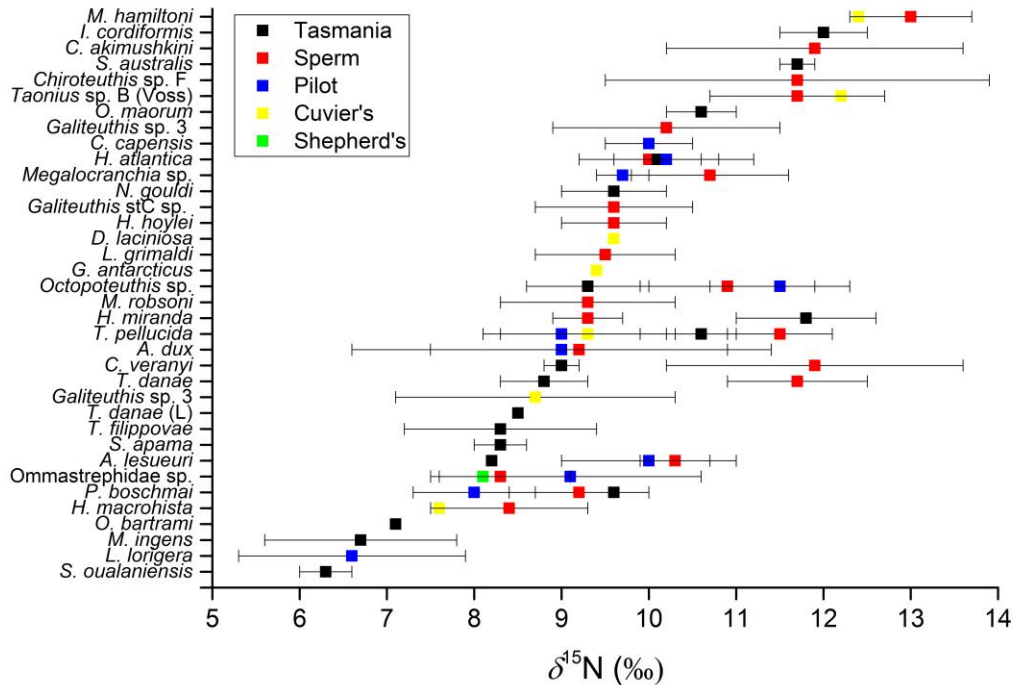


Figure 6.3. Mean \pm SD beak $\delta^{15}\text{N}$ values of cephalopod species consumed by toothed whales (Sperm *Physeter macrocephalus*, Pilot, *Globicephala melas edwardii* Cuvier's *Ziphius cavirostris* and Shepherd's beaked whales *Tasmacetus shepherdi*) from this study as well as all cephalopod species caught in waters around Tasmania. *Galiteuthis* stC sp., *Galiteuthis* stC sp. (Imber); *Galiteuthis* sp. 3, *Galiteuthis* sp. 3 (Imber); *Chiroteuthis* sp. F, *Chiroteuthis* sp. F (Imber)

overlap in prey species and foraging habitat, the foraging niches of the sperm, pilot and Cuvier's whales appear to be relatively separated (Figure 6.1). This reinforces the likelihood that both the pilot whales and the Cuvier's beaked whale are eating other unknown prey.

The similarity between the $\delta^{13}\text{C}$ values of the cephalopods from the stomachs of the stranded whale predators and the cephalopods caught in Tasmanian waters suggests that the odontocetes in this study had been foraging in subtropical waters

(Figure 6.4). $\delta^{13}\text{C}$ values can only give a broad indication of habitat. For example, *Moroteuthis ingens* from New Zealand had similar $\delta^{13}\text{C}$ values to *M. ingens* caught in

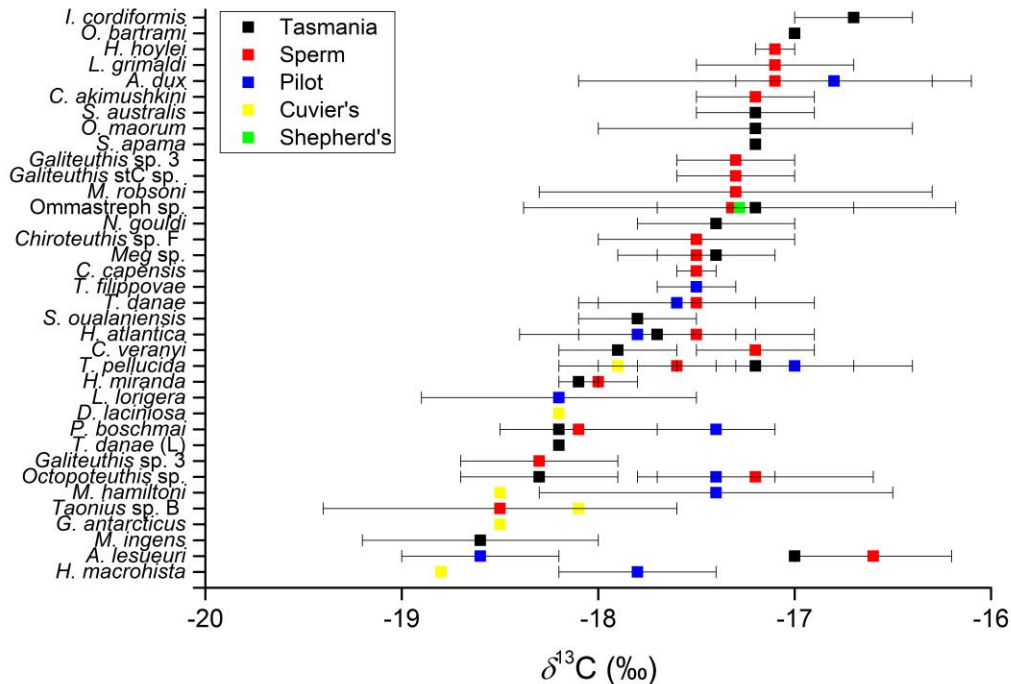


Figure 6.4. Mean \pm SD beak $\delta^{13}\text{C}$ values of cephalopod species consumed by toothed whales (Sperm *Physeter macrocephalus*, Pilot, *Globicephala melas edwardii* Cuvier's *Ziphius cavirostris* and Shepherd's beaked whales *Tasmacetus shepherdi*) from this study as well as all cephalopod species caught in waters around Tasmania. *Taonius* sp. B, *Taonius* sp. B (Voss); *Galiteuthis* sp. 3, *Galiteuthis* sp. 3 (Imber); *Chiroteuthis* sp. F, *Chiroteuthis* sp. F (Imber); *Ommastreph* sp., *Ommastrephidae* sp.; *Galiteuthis* stC sp., *Galiteuthis* stC sp. (Imber)

Tasmanian waters and *Sthenoteuthis oualaniensis* caught in waters off eastern Australia also had similar values to pelagic squid caught in Tasmanian waters. Since the small and large scale migration patterns are not clearly understood for many of the toothed whale species that strand in Tasmanian waters cephalopod habitat can only be inferred. However, tagged sperm whales have been observed moving longitudinally in waters south of Australia over to New Zealand (Brown 1981 cited in Evans & Hindell 2004a). Despite this, many of the cephalopod species

documented in the stomach contents of the whale species have been previously observed as by-catch in Tasmanian waters (G. Jackson personal communication).

An overview of the cephalopod species represented in the stomachs of the toothed whales in this study supports the idea that competition between whales and fisheries (at least as it relates to cephalopods) is unlikely to pose a major threat to the status of fisheries in this region. Many of the cephalopods consumed are gelatinous or ammoniacal and not subject to commercial fisheries. Moreover, any remaining species are not being commercially harvested, with the one exception being Ommastrephidae sp. that were consumed by most toothed whales in this study although not in substantial numbers. The only ommastrephid being commercially harvested in Tasmanian waters is the shelf species *Nototodarus gouldi* (Stark et al. 2005). This is a potential important prey item for pilot whales but not for sperm whales and Cuvier's beaked whales that appear to forage in deeper waters. However, in the Tasmanian region prey competition with fisheries does not appear to be a major threat to whale population recovery.

The recovery of whale populations in Australia from years of overexploitation due to industrial whaling has been sporadic and incomplete. It appears it is predominantly the baleen whales as opposed to the toothed whales that are currently more highly endangered or vulnerable (e.g. blue whale *Balaenoptera musculus*, southern right whale *Eubalaena australis*, sei whale *Balaenoptera borealis*, fin whale *Balaenoptera physalus* and humpback whales *Megaptera novaeangliae*) (<https://www.environment.gov.au>) which do not consume cephalopods. Although all cetaceans are now protected in Australian waters it is likely that to ensure continued whale recovery a multi-species ecosystem based management system would appear to be needed. This kind of management serves to protect interacting species as opposed to a single species in isolation (Boyd 2002, Williams et al. 2011). For example, prey requirements (e.g. squid) would be taken into account when managing and protecting toothed whale species such as sperm whales. The toothed whales examined in this study do not appear to be highly specialized in their dietary

requirements like the southern resident killer whale in the northeastern Pacific (*Orcinus orca*) whose primary prey is Chinook salmon (*Oncorhynchus tshawytscha*) (Williams et al. 2011). While the whales in this study appear to specialize on oceanic cephalopods, there is some flexibility in the range of species consumed. Given the central and significant role that cephalopods play in the marine ecosystem (Coll et al. 2013) this would appear to be an important step for continuing to see a recovery in whale populations. While fisheries do not heavily target cephalopods consumed by the toothed whales in this study, they have been product of deep-sea fishery by-catch in Tasmanian waters (G Jackson, personal communication).

Cephalopod community

Overall a total of 31 cephalopod species and two mixture of species (*Ommastrephidae* sp. and *Octopoteuthis* sp.) were obtained either through by-catch of a deepwater Tasmanian fishery, commercial fisheries or from odontocetes used as biological samplers in this study. The overall relatively restricted values of $\delta^{13}\text{C}$ ranged from -18.6‰ and -18.8‰ for species such as *Histioteuthis macrohista* and *Moroteuthis ingens*, respectively (with lower $\delta^{13}\text{C}$ values reflective of an oceanic habitat) to -16.6‰ , -16.7 and -16.8‰ , respectively for *Ancistocheirus lesueuri*, *Idioteuthis cordiformis* and *Architeuthis dux* respectively (with higher $\delta^{13}\text{C}$ values more representative of a deepwater or benthopelagic habitat). Inshore or neritic cephalopod species such as *Sepioteuthis australis*, *Sepia apama* and *Octopus maorum* had more enriched $\delta^{13}\text{C}$ values similar to deepwater species which is indicative of the inshore/offshore and pelagic/demersal ^{13}C contributions to dietary intake (Hobson et al. 1994, Cherel & Hobson 2007). However, all species reflected a subtropical water isotopic signature (Figure 6.4).

The $\delta^{15}\text{N}$ values of all cephalopod species (excluding those used as comparison species i.e. *S. oualaniensis*, *Histioteuthis eltaninae*, *Slosarczykovia circumantarctica* *Galiteuthis glacialis*) ranged from the small *Lycoteuthis lorigera* (6.6‰) to the colossal squid *M. hamiltoni* (13.0‰). The $\delta^{15}\text{N}$ values spanned approximately three distinct trophic levels similar to that found in Kerguelen waters (Cherel & Hobson

2005). Within species some squid such as *A. dux* and *Histioteuthis atlantica* had similar $\delta^{15}\text{N}$ values whether sperm whales or pilot whales consumed them (Figure 6.3). However, other species such as *Teuthowenia pellucida*, *A. lesueuri* and *Pholidoteuthis boschmai* differed in both their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values depending on which whale species ingested them (Figure 6.3, 6.4). Although cephalopod communities are not trophically size-structured (based on $\delta^{15}\text{N}$ values), individual cephalopod species are size-structured according to their trophic level. An increasing dorsal mantle length normally results in a gradual increase in $\delta^{15}\text{N}$ values (Chapter 2). Some whale species tend to consume prey of a particular size (MacLeod et al. 2006b), which may result in varying $\delta^{15}\text{N}$ values for the cephalopod species consumed due to the lack of trophic size structure between species. Furthermore, some cephalopod species may be more widespread in their distribution within subtropical waters accounting for varying $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for a species.

One of the most surprising features of the cephalopod community was the high $\delta^{15}\text{N}$ values and presumably high trophic level of some species. *M. hamiltoni* had the highest values in this study exceeding that of the sperm whale and killer whale (see Davenport & Bax 2002). *M. hamiltoni* is also a top predator in Kerguelen waters (Cherel & Hobson 2005). Other cephalopod species were also higher order predators in this ecosystem such as *T. danae*, *Chiroteuthis veranyi*, *Chiroteuthis* sp. F, *Cycloteuthis akimushkini* and the cranchiids, *Teuthowenia pellucida*, *Megalocranchia* sp. and *Taonius* sp. B (Voss). All these cephalopod species had $\delta^{15}\text{N}$ values exceeding that of the sperm whales, which had the highest $\delta^{15}\text{N}$ values of all whales in this study. Many of the same species were also higher order predators in other cephalopod communities (Cherel & Hobson 2005, Cherel et al. 2009a). This study confirms that cephalopods are able to exploit and prey upon organisms from all trophic levels and that they themselves are predominantly middle to higher order predators. Moreover, their importance in the marine ecosystem is reiterated, providing and transporting energy up the food chain (Coll et al. 2013).

Future research

Stable isotope analysis is a powerful technique that can characterize community metrics such as the assemblage of species, resource partitioning and predator-prey interaction (Crawford et al. 2008). Since this methodology allows for sampling of marine mammal predators through minimally invasive procedures such as blood samples or dart biopsies, sampling of both can be practical and viable (Ramos & González-Solís 2012). As inadequate sampling of the diet of the consumer often makes for equivocal interpretation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, more isotopic data of potential prey across a wide spectrum should be undertaken (Crawford et al. 2010, Young et al. 2015a). Furthermore, as comparisons can only be made within ecosystems with analogous $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ baselines (Lorrain et al. 2010) sampling should be done on the same spatial and temporal scale as much as possible. One of the areas with a high potential for error is the use of erroneous discrimination factors. The discrimination factors used in many studies have been based on only a few species. Therefore, there is a continued need for more controlled studies of marine mammal species of interest to be fed an isotopically distinct diet whereby the tissue turnover and metabolic routing to specific tissues can be assessed (Crawford et al. 2010, Layman et al. 2010, Giménez et al. 2016).

A continuation of the use of marine predators as biological samplers to understand the foraging ecology of the consumer and well as the trophic dynamics of the prey is essential. This is particularly pertinent to cephalopods since ecological models have highlighted their importance as relevant organisms in the food web at both local and regional scales with potential top-down impacts on their prey. Cephalopods have very plastic lifestyles including dietary behaviour resulting in substantial trophic width, encompassing lower to higher order levels (Coll et al. 2013). Their importance to apical predators is undisputed and more comparisons between the isotopic value of the consumer and stomach contents of the same individuals will provide information not afforded by stomach content analysis alone. More predator-prey studies incorporating these methodologies are also encouraged along with other biochemical techniques such as fatty acid analysis, DNA molecular analysis

and heavy metal analysis (Bustamante et al. 1998, Herman et al. 2005, Fontaine et al. 2015, Pethybridge et al. 2015, Watt & Ferguson 2015, Xavier et al. 2016). Understanding predator-prey interactions are essential to determining potential influences on the structure and function of an ecosystem (Heithaus et al. 2008).

The use of marine mammals as environmental biomonitors requires increased awareness of the spatial and trophic dynamics of species so that factors affecting their viability and resilience are understood (Ramos & González- Solís 2012). How cetaceans respond to changes in the availability and quality of their prey is possibly linked to the cost of living for an individual species. Species that depend on high quality food due to their high cost of living are more likely to be severely impacted than those that flourish on low quality diets. Those that can thrive on low quality diets will likely have more prey options (Spitz et al. 2012). Therefore, determining predator-prey relationships for cetaceans is paramount if recovering populations are to be sustained globally.

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